MEDICINAL PLANTS USED IN THE MANAGEMENT OF DEPRESSIVE ILLNESSES: ANTIDEPRESSANT ACTIVITY OF METHANOL STEM BARK EXTRACT OF *ADANSONIA DIGITATA* L. IN MICE

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FACULTY OF PHARMACEUTICAL SCIENCES,
AHMADU BELLO UNIVERSITY, ZARIA
NIGERIA

AUGUST, 2018

DECLARATION

I declare that the work in this thesis entitled ‘’Medicinal Plants used in the Management of Depressive Illnesses: Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia Digitata* L. in Mice’’ has been performed by me in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The information derived from the literature have been duly acknowledged in the text and a list of references provided. No part of this thesis has been previously presented for another Degree or Diploma at this or any other University.

Aishatu SHEHU		
Name of the student	Signature	Date

CERTIFICATION

This thesis entitled **MEDICINAL PLANTS USED IN THE MANAGEMENT OF DEPRESSIVE ILLNESSES: ANTIDEPRESSANT ACTIVITY OF METHANOL STEM BARK EXTRACT OF *ADANSONIA DIGITATA* L. IN MICE** by Aishatu SHEHU meets the regulations governing the award of the degree of Doctor of Philosophy 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 Allah, then to my loving daughter Ummita

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ABSTRACT

Depression is a heterogeneous mood disorder that has been treated with a number of synthetic drugs. These drugs have adverse effects and delayed onset of action that compromise their therapeutic benefits. This makes it worthwhile to search for new antidepressant agents with proven efficacy and favourable benefit-to-risk ratio. Medicinal plants have enjoyed wide patronage among local people in the management of neuropsychiatric disorders including depression. The study therefore aimed at establishing a collection of medicinal plants used in the management of depressive illnesses by the traditional medical practitioners of Zaria, Nigeria, as well as provide scientific basis for their ethnomedical use in the management of depression and determine the antidepressant activity of the most promising of the collected plants and its possible mechanism of action. An ethnobotanical survey was conducted in December, 2015 in Zaria, Nigeria in which data were collected by interviewing traditional medical practitioners in Zaria with the aid of a questionnaire. Plant specimens were collected along the line, subsequently dried and mounted. They were taken for identification and authentication in the Herbarium Section of Botany Department, Ahmadu Bello University, Zaria where specimen vouchers were documented. Information on sources, safety, methods of preparation and administration, identity, local and botanical names of ten medicinal plants used in the management of depression by traditional medical practitioners were obtained. Nine of the medicinal plants were further collected and then extracted with methanol using soxhlet apparatus method of extraction. Thin layer chromatographic finger printing of the medicinal plants extract was conducted, followed by acute toxicity (LD_{50}) studies using oral OECD 420 guidelines. Antidepressant activity of all the medicinal plant extracts was evaluated using tail suspension test (TST), followed by test for motor co-ordination deficit and stimulant activity using beam walking assay

(BWA) and open field test (OFT) respectively. The most promising extract, methanol stem bark extract of *Adansonia digitata* was further subjected to forced swim test (FST), novel object recognition test (NORT) and chronic unpredictable mild stress (CUMS). Following the CUMS, the effect of extract was also assessed on sucrose preference test (SPT), OFT and TST. The possible mechanism(s) of action of *Adansonia digitata* extract was also determined. The plants used by the traditional practitioners in Zaria included *Ficus platyphyllla, Caralluma dalzielli, Adansonia digitata, Acacia seyal, Tapinanthus dodeinofolius, Tapinanthus globiferus, Senna occidentalis, Olax manni, Combretum micranthum* and *Pancrantium africanum*. The chromatographic profile of the extracts of these medicinal plants showed the presence of steroids, tannins, flavonoids, alkaloids, triterpenes, saponins in all the plants and anthraquinones in some of the medicinal plants. The oral median lethal doses of the methanol extracts of *F. platyphylla, C. dalzielli* and *O. manni* were found to be greater than 2000 mg/kg while that of others were greater than 5000 mg/kg. The methanol extracts of all the plants significantly (p<0.05) decreased the duration of immobility at all tested doses in the TST. The methanol extracts of the medicinal plants did not significantly increase the number of lines crossing and the number of foot slips in the OFT and BWA respectively. The methanol stem bark extract of *A. digitata* (MEAD) significantly and dose dependently decreased the duration of immobility in FST, with no alterations on cognition in the NORT when acutely administered. Following the CUMS, MEAD significantly (p<0.05) and dose dependently reversed weight loss of mice, the inhibited locomotor behaviour in the OFT, decreased sucrose consumption in SPT, reduced the duration of immobility in TST and reversed the impaired cognition in the NORT. The antidepressant activity produced by MEAD was significantly (p<0.05) reversed by sulpiride (D_2/D_3 receptor antagonist), metergoline (5-HT_1 antagonist), cyproheptadine (5-HT_2 antagonist), prazosin (α_1 adrenergic antagonist),

yohimbine (α_2 adrenergic antagonist) and augmented by L-NNA (Nitric oxide synthase inhibitor) and atropine (muscarinic antagonist). MEAD significantly ($p<0.05$) and dose dependently increased the levels of brain derived neurotrophic factors (BDNF), decreased the levels of plasma cortisol, increased the levels of total superoxide dismutase (SOD) activity and decreased plasma malondialdehyde (MDA) levels in mice following CUMS. The findings from this study provide pharmacological rationale for the folkloric use of the medicinal plants in the management of depression by traditional medical practitioners in Zaria, Northwestern Nigeria. The methanol stem bark extract of *Adansonia digitata* possesses significant antidepressant activity in both acute and CUMS model of depression, possibly mediated via the monoaminergic, nitritergic, metabolic, neurotrophic and neuroendocrine systems.

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LIST OF ABBREVIATIONS AND SYMBOLS

ACTH	Adrenocorticotrophic hormone
AMPA-R	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
AVP	Arginine vasopressin
BDNF	Brain Derived Neurotrophic Factor
CREB	cyclic AMP receptor element binding
CRH	Corticotrophin releasing hormone
CUMS	Chronic Unpredictable Mild Stress
ELISA	Enzyme Linked Immunosorbent Assay
ERK	Extracellular Signal Regulated Kinase
FST	Forced Swim Test
GRs	Glucocorticoid receptors
HPA	Hypothalamus Pituitary Adrenal axis
Kg	Kilogram
MARCKS	Myristoylated alanine-rich C Kinase Substrate
MDA	Malondialdehyde
MDD	Major depressive disorder
MEAD	Methanol Stem Bark Extract of *Adansonia digitata*
Mg	Milligram
Mrna	messenger Ribonucleic Acid
MRs	Mineralocorticoid receptors
Mtor	mammalian Target of rapamycin
NMDA	N- methyl D Aspartate
NORT	Novel Object Recognition Test

NPCs	Neural Progenitor Cells
NSCs	Neural Stem Cells
OECD	Organization for Economic Coperation and Development
OFT	Open Field Test
PDZ	Post synaptic density protein; Drosophilia disc large turmor suppressor, Zonula occludens-1 (initials of these first three proteins that formed PDZ domain)
PSD-95	Post synaptic density protein 95
S	Seconds
SNRIs	Selective Noradrenaline Reuptake Inhibitors
SOD	Superoxide Dismutase
SPT	Sucrose Prefence Test
SSRIs	Selective Serotonin Reuptake Inhibitors
TCAs	Tricyclic Antidepressants
TLC	Thin Layer Chromatography
TRD	Treatment Resistant Disease
TrKB	Tropomysin receptor kinase B
TST	Tail Suspension Test
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The use of medicinal plants as remedies is common and widespread in Nigeria (Olajide, 2003) and the use of plants by man in curing illness has a long history (Grabley and Thiericke, 1999; Petrovska, 2012). It has been stated severally that traditional medicine often provides culturally familiar techniques that treat both physical and spiritual conditions (Pachter, 1994). The word *ethno* means the way human beings see the universe. It is used as a prefix in disciplines like botany and pharmacology (Erinoso and Aworinde, 2012). Ethnobotany is the study of useful plants prior to and eventful domestication. It is based on the knowledge of plants by local people and their usefulness as understood by people of a particular ethnic group (Igoli *et al*., 2005). Many researchers have conducted ethnobotanical surveys among African tribes and in different parts of the world in search of plants with antibacterial, antifungal and antiviral properties (Khan and Rashid 2006, Ajaiyeoba *et al*., 2006). Ethnobotanical surveys are important in determining the social, cultural and economic factors as regards to ideas and action concerning health and illness. It also helps in gathering information on type of disease and health problems, prevalent among people of certain localities (Lawal, 2010).

Disorders of mood are often called affective disorders. The word ‘affect’ refers to the external display of mood which is an emotion felt internally (Philip *et al*., 2013). Depression and mania are mostly seen as opposite ends of affective spectrum and both

disorders are poles. The two poles can occur simultaneously termed as mixed mood state. The most common and easily identified mood disorder is depression (Stein, 2008).

Depressive disorder is a long term relapsing condition associated with high levels of disability and mortality. It has a neurobiological basis and is associated with functional and structural brain abnormalities (Stein, 2008). Depression is a highly prevalent psychiatric disorder with a life time risk close to 20 %, associated with high level of morbidity and mortality (Abou-Saleh *et al*., 2017). Although depressed patients are at high risk of serious physical health problems such as coronary artery diseases, diabetes and worsening of the prognosis of other medical conditions. Depression remains a neglected problem in palliative care provision across Africa (AR, 2013). A new report has found that African countries have some of the highest rates of diagnosed clinical depression in the world. Researchers at University of Queensland Australia have found that some countries in Africa have over 7% of their population diagnosed with clinical depression (Ferrari *et al*., 2013). Although, measuring, monitoring and managing depression is a central element of palliative care, the nature of the support patients need varies vastly. The understanding of depression and other mental health issues vary across Africa for because of high prevalence of the disorders in which remains unclear (AR, 2013).

In Nigeria, depression was found to be common among people living with HIV/AIDS (Chikezie *et al.,* 2013). It is associated with poor HIV treatment adherence and higher mortality rates. Depression was also observed to be prevalent in females than in males during an overview of the socio-cultural and psychiatric aspects of women's reproductive

health in Nigeria (Adewuya *et al*., 2006). Furthermore, depression has been reported to be prevalent among civil servants in Nigeria (Yusuf and Adeoye, 2011), this may not be unconnected with the poor workers' conditions and environments (Follmer and Jones, 2017). A review on the prevalence of depression among university students in Nigeria revealed prevalence ranged from 10-85% (Ibrahim *et al.*, 2013). Poor academic performance (Yusoff, 2013), addictive behavior (Adewuya, 2006) and stressful traumatic life events (Buchandent, 2012; Adewuya *et al*., 2016) are factors linked to increase prevalence of depression among Nigerian students and adults.

Depression is a common illness and at some point in life, around one in every five women and one in every ten men will suffer depression. At any given time, one in every twenty adults is experiencing a serious major depression (Umoh *et al*., 2008). In any one year, about three in every ten employees have mental health problems, and depression is one of these most common problems (Miaomiao *et al*., 2017). It makes sufferers less productive at work and is responsible for high rates of sick leaves, accidents and staff turnover (Yingxiao *et al*., 2017). Depressions have a substantial effect on employees by interfering with their ability to do their jobs as well as their life activities like sleep, eat, study and pleasures (WHO, 2017).

1.2 Statement of Research Problems

Depression affects 10% of the global population (Brent, 2016; Calvo-Perxas *et al*., 2016) and is estimated to rank first among global burden diseases by 2030 (Filho *et al*., 2016). The development of any society and organization depends on its human and natural resources (Murphy and Byrne, 2012). Problems of depression are common in the general

population but more pronounced in working class due to expectations placed on them at work, which tend to confer negative effects on their effectiveness and productivity (Stynen *et al*., 2015). Although, very effective treatments are available for depression, only about one third of those who are depressed receive these treatments (Duric *et al*., 2017).

Depression affects approximately 19 million Americans, or 9.5% of the population in any given one-year period. At some point in their lives, 10-25% of women and 5-12% of men will likely become clinically depressed. It is estimated that depression exerts an economic cost of over 30 billion dollars each year (Ghulam *et al*., 2014). Depression causes suffering to the depressed individuals and also great difficulty for their family and friends who often do not know how to help (Smith *et al*., 2012).

Clinical depression affects all aspects of a person's life. It impairs ability to sleep, eat, work and get along with others. It damages self-esteem, self-confidence and ability to accomplish everyday tasks (Kimberel *et al*., 2016). People who are depressed find daily tasks to be a significant struggle, they get tired easily, yet cannot get a good night sleep. They have no motivation and lose interest in activities that were once enjoyable (Oberholzer *et al*., 2017).

Suicide is another possible complication of depressive illness in combination with other risk factors because suicidal thoughts and behavior can be symptoms of moderate to severe depression (Citrome, 2014). Reports have shown that up to 15% of clinically depressed people die by suicide (Paul *et al*., 2012a). The majority of suicide attempts are

expressions of extreme distress that need to be addressed, and not just harmless bid for attention (Pasquini *et al*., 2014). Depression has been reported to be strongest risk factor for suicidal attempts in both adults and youth (Duda *et al*., 2016). Antidepressants such as the selective serotonin reuptake inhibitors (SSRIs) has been realized to circulate throughout the body when administered and interfere with important diverse processes regulated by serotonin and confer harmful effects on other body processes like digestion, sexual function and abnormal bleeding (Khan *et al*., 2018).

Most of the antidepressants in existence are slightly more effective than a placebo (Turner *et al*., 2008; Berger *et al*., 2009) with treatment over months before observable efficacy, which has frequently resulted in full blown relapse (McGrath *et al*., 2008, Bockting *et al*., 2008; Best *et al*., 2010). Antidepressants currentlty in use have been reported to be associated with a lot of toxicities and adverse effects (Khan *et al*., 2018) such as neuronal damage and death in rodents (Tanrikut *et al*., 2010), involuntary repetitive movements in humans that results in parkinsonian symptoms (Cheng *et al*., 2010; Paul *et al*., 2012) and tardive dyskinesia (Andrews *et al*., 2011; Fava and Offidani, 2011). Some of the existing antidepressants are implicated in the increase risk of breast cancer (Jackson, 2009; Ganzel *et al*., 2010; Cosgrove and Krimsky, 2012) and their prolonged use is associated with a 70% increase in the risk of mild cognitive impairment and probable dementia (Goveas *et al*., 2011). They were also found to negatively impact sperm structure, volume and mobility resulting in serious sexual dysfunction (Ridout *et al*., 2016). Antidepressants prescribed to older people tend to increase risk of many negative effects like falling, bone fracture, hyponatremia development and death (Coupland *et al*., 2011). Some existing antidepressants are associated with increase risk of cardiovascular events by elevating platelet level and enhancing pro-aggregating processes (Flook *et al*., 2010).

Despite the wide treatment options for depression only approximately one third of patients achieve a temporary dimunition of the severity of the disease or pain (Strawbridge *et al*., 2017). Furthermore, the appearance of treatment-resistant depression (TRD) to classic antidepressant and delay in the onset of action for longer periods indicates additional pathogenesis to depressive episodes, increased functional impairment, mortality, morbidity and recurrent or chronic episodes in the long term (Oberholzer *et al*., 2017). In view of all these, new antidepressant agents need to be developed with better efficacy and safety profile.

1.3 Justification for the Study

The use of medicinal plants for the treatment of depression is a common practice in many African countries including Nigeria. Despite the immense technological advancement in modern medicine, over half of the world's population rely on traditional medicine (herbal drugs) for their daily health care needs (Raphael, 2011). According to world health organization (WHO), over 80% of people in developing countries like Nigeria depends on herbal medicines because they are more affordable options to pharmaceutical drugs. This gave a growing popularity of scientific interest in herbal remedies (Ansari and Inamdar, 2010). Additionally, scientific evidence of efficacy of St. John's wort used in a clinical trial has been reported useful for mild depression and this formed basis for screening other medicinal plants for antidepressant properties. Recently, a growing number of herbal medicines such as *Lavendula aungustifolia* was chosen as alternative therapies for depression (Kute *et al*., 2018). The fact that many pharmaceutical products such as quinine and digitoxin are of botanical origin also strengthen the screening of medicinal plants for possible novel compounds.

Moreover, the high cost of acquiring synthetic drugs, shortages, side effects and toxicities, limitations in use and ineffectiveness especially due to development of desensitization and dependence caused by antidepressants drugs (Khan *et al*., 2018) had led to the investigation of the antidepressant effect of medicinal plants used by traditional healers to treat depression most especially among Hausa tribes in Nigeria.

Additionally, herbal drugs were found to offer advantages in terms of safety and tolerability, possibly also improving patients' compliance (Kamalipour *et al*., 2008). This has given popularity to medicinal plants over pharmaceuticals. However, studies have shown that not all natural products are safe, even poisons can be of natural origin. Thus the need to screen the toxicity profile of the reported medicinal plants used in the management of depression among Hausa tribes of Kaduna state, Nigeria. The fact that Nigeria has established National and State Traditional medicine boards for regulation of herbal medicine practice to promote cooperation and research (Awodele *et al*., 2014) is another challenging factor why pharmacological activities of medicinal plants should be validated and documented.

The validation of folkloric claims of therapeutic efficacy of medicinal plants helps supports tropical conservations of plant resources.

1.4 Aim

The aim of this research was to evaluate the antidepressant activities of medicinal plants used in the management of depression by traditional medical practitioners in Zaria, Nigeria.

1.5 Specific Objectives

1. To establish a collection of medicinal plants used in management of depression by traditional medical practitioners in Zaria, Nigeria.
2. To establish the phytochemical constituents present in the plants.
3. To determine the acute toxicity (LD_{50}) profile of the medicinal plants in mice.
4. To evaluate the antidepressant activities of the surveyed medicinal plants in mice.
5. To determine the effect of the most active plant on stress induced depression in mice.
6. To determine the effect of most active plant on anhedonia in mice.
7. To establish the involvement of neuroendocrine and neurotrophic systems in the antidepressant activity of the most active plant in mice.
8. To assess the involvement of oxidant and antioxidant system in the antidepressant activity of the most active plant in mice.
9. To establish the possible involvement of neurotransmitter system in the mechanism of action of the most active plant in mice.

1.6 Theoretical Framework

1.6.1 Ethnobotanical survey

Ethnobotanical survey is usually conducted in order to obtain relevant information about medicinal plants used in the treatment of diseases in a particular society. Data are usually collected based on semi structured questionnaires and authentication follows in the herbarium (Erinoso and Aworinde, 2012).

1.6.2 Preliminary phytochemical screening

Basic phytochemical screening is designed to detect the presence or absence of some classes of plant metabolites by subjecting them to reaction with reagents that could yield

observable colored products. Some of the reactions involved formation of complexes between the organic metabolites and heavy metals resulting to appearance of colored precipitate (Sofowora, 1993; Evans and Trease, 2008).

1.6.3 Acute toxicity studies

Toxicity refers to the ability of chemical agents to cause injury. Chemical substances may evoke one or both toxic effects. The first is in acute effect which occurs shortly after contact with a single dose of poison. The second is chronic effects which occur however, when an organism is exposed to repeated small and non-lethal dose of potentially harmful substances. Toxicity is categorized as acute, subacute and chronic (OECD, 420).

1.6.4 Forced swim test

Behavior despair was proposed as a model to test for antidepressant activity. It was suggested that mice or rats forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviors of immobility. This behavior reflects a state of despair which can be reduced by several agents that are therapeutically effective in human depression (Porsort *et al*., 1978).

1.6.5 Tail suspension test

Tail suspension test has been described as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioral despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail (Steru *et al*., 1985).

1.6.6 Open field test

An open field test utilizes a large cubic box, usually measuring 72 cm long × 72 cm wide × 36 cm high. The top of the cube is typically left uncovered. An animal is placed in the middle of the bottom surface, and its movements are recorded over the course of minutes to hours as it moves around and explores its environment. After the experiment is completed, the movements of the animal over time is analyzed. This assay can measure horizontal activity, time spent in various regions of the open field, and the total distance travelled. This assay can also be used to measure anxiety behaviours (Rex *et al*., 1998).

1.6.7 Novel object recognition test

The object recognition test (ORT) is a commonly used behavioral assay for the investigation of various aspects of learning and memory in mice (Lueptow, 2017). The ORT is fairly simple and can be completed over 3 days: habituation day, training day, and testing day. During training, the mouse is allowed to explore 2 identical objects. On test day, one of the training objects is replaced with a novel object. Because mice have an innate preference for novelty, if the mouse recognizes the familiar object, it will spend most of its time at the novel object. Due to this innate preference, there is no need for positive or negative reinforcement or long training schedules (Cordeira *et al*., 2018).

1.6.8 Chronic unpredictable mild stress

A proposed complex relationship exists between stressful situations, mind and body's reaction to stress and the onset of clinical depression. Thus, evidences showed that the chronic administration of unpredictable stress is an appropriate model for the pre-clinical evaluation of antidepressants (Bhutani *et al*., 2008).

1.7 Statement of Research Hypothesis

Methanol extracts of medicinal plants used in the management of depression by traditional medical practitioners in Zaria, Nigeria possess antidepressant activities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Depression

Depression is a heterogeneous condition characterized by multiple symptoms and subtypes (Stein, 2008; Miaomiao *et al*., 2017). It is a widespread, devastating illness affecting approximately 17% of the population at some point in life, resulting in enormous personal suffering and economic loss (Ronald, 2014; Abou-Saleh *et al*., 2017). Depression is a long term relapsing condition associated with high level of disability and mortality. It has a neurobiological basis and associated with functional and structural brain abnormalities (Eleni, 2012). Depression is a disorder affecting mood and general outlook sometimes called 'the blues'. It is characterized by a loss of interest in activities or feeling sad and down. It is a serious medical condition and people usually don't get over a depression state (Dandekar *et al*., 2018).

2.2 Pathophysiology of Depression

Depression is a complex and heteregenous disease that several basic and clinical researches have been conducted in order to elucidate the pathophysiology. Significant progress has been made to identify brain regions that control emotion, mood and anxiety. So also, the neurochemical and cellular alterations underlying depression and stress related disorders have been studied (Ronald, 2014, Carneiro *et al*., 2016). It is evident that stress and depression cause neuronal atrophy and damages in brain regions controlling emotion and mood, resulting in disconnection and loss of function (Dunan and Monteggia, 2006; Krishnan and Nestler, 2008). The damages include reduction in number of spine synapses, which is the key point of connection between neurons (Khan *et al*., 2018). In some cases, there is evidence of hypertrophy and increased function of

other brain regions which contribute to dysregulation of mood and anxiety (Chandrasekhar *et al*., 2017). In addition to the role of increased activity of hypothalamic pituitary adrenal axis (HPA) as hall mark of stress responses, disruptions of neurotrophic or growth factors have been said to be involved in the atrophy and loss of neurons, in response to stress and depression (Drevets *et al*., 2008).

Another study supports the hypothesis that alterations of synaptogenesis and neuronal plasticity resulting in functional disconnections underlie the pathophysiology of depression (Vishnu *et al*., 2017). It was observed that negative factors that reduce synaptogenesis in a circuit specific manner (e.g stress, inflammatory cytokines, glucocorticoids) increase risk to or cause depression (Kang *et al*., 2012; Ozkartal *et al*., 2018). Synaptogenesis depends upon the assembly of new synapses and the disassembling of old synapses by β- adducin. Adducins are membrane skeletal proteins that cap the growing ends of actin filaments and promote their association with spectrin, another cytoskeletal protein to create stable and integrated cytoskeletal networks. Actins have a variety of roles in synaptic functioning (Jesulola *et al*., 2018). In pre-synaptic neurons, actins are involved in synaptic vesicle recruitment and vesicle recovery following neurotransmitter release. In post synaptic neurons, they can influence dendritic spine formation and retraction as well as α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA-R) insertion and removal. At their C- terminus, adducins possess a myristoylated alanine- rich C kinase substrate (MARCKS) domain which regulates their capping activity. Brain derived neurotrophic factor (BDNF) can reduce capping activities by upregulating PCK, which can bind to adducins MARCKS domain, inhibit capping activity, and promote synaptogenesis through dendritic spine growth, disassembly and other activities (Maes *et al*., 2012). Exposure to stress and stress hormone corticosterone has been shown to reduce the expression of BDNF and persistent

exposure leads to an eventual atrophy of the hippocampus and other limbic structures in humans suffering from chronic depression. Apart from decreasing neuronal alterations and synapse number, stress is reported to exert significant impact on the production of new neurons and glia (Hasler, 2010).

Another mechanism that has been linked to atrophy and loss of neurons associated with stress is the disruption of neurogenesis in the dentate gyrus of adult hippocampus (Roozendaal *et al*., 2009). BDNF plays a significant role in neurogenesis. It promotes protective pathways and inhibits damaging pathways in the neural stem cells (NSCs) and neural progenitor cells (NPCs) that contribute to the brain neurogenic response by enhancing synaptic plasticity and cell survival (Duman and Voleti, 2012; Filho *et al*., 2016). This becomes evident following suppression of Tropomysin receptor kinase B (TrKB) activity. TrKB is a receptor for BDNF (Jang *et al*., 2009). TrKB inhibition results in a 2-3 folds' increase in cortical precursors displaying EGFP-positive condensed apoptotic nuclei and a 2-4-fold increase in cortical precursors that stained immunopositive for cleaved caspase-3. So also, local interaction of BDNF with the TrKB receptor on a single dendritic segment is able to stimulate an increase in postsynaptic density protein 95 (PSD-95) trafficking to other separate dendrites as well as the synapses of locally stimulated neurons. PSD-95 localizes the actin remodeling GTpases, Rac and Rho to synapses through the binding of its PDZ domain to kalirin (PDZ is an initialism combining the first three proteins that formed it; post synaptic density protein, Drosophilia disc large turmor suppressor and zonula occludens-1), increasing the number and size of spines. Thus, BDNF induced trafficking of PSD-95 to dendrites stimulates actin remodeling and causes dendritic growth in response to BDNF (Michael and Diego, 2014). Stress was found to impact the expression of BDNF resulting in decrease level of messenger RNA (mRNA) and protein in the hippocampus and prefrontal cortex

(Krishnan and Nestler, 2010; Audet and Anisman, 2013). There is also a decrease in blood levels of BDNF in depressed patients (Schmidt *et al*., 2011). Brain derived neurotrophic factor deletions has been extrapolated to increase vulnerability to depressive behaviour in rodents, expressed as despair, anxiety and anhedonia in depressive models (Yu *et al*., 2012; Ozkartal *et al*, 2018). Stress has been demonstrated to result in reduced BDNF expression, thus loss of BDNF or its mutation that reduces release, have negative effects on dendrite morphology and behaviour (Jun *et al*., 2016; Jiang *et al*., 2017).

Brain imaging have revealed key structures involved in the regulation of mood and depression which include the prefrontal cortex, hippocampus, cingulate cortex, amygdala and basal ganglia (Savitz and Drevets, 2009). Studies such as blood flow and functional imaging have indicated region with reduced prefrontal cortex and hippocampus in disrupted connectivity of cortical and limbic depression circuitry (Khan *et al*., 2018). This disruption in connectivity between the prefrontal cortex and amygydala, and also in the ventral striatum contributed to reduced motivation and reward in depression. This manifests as sleeping, eating and libido disruptions (Bansar *et al*., 2007; Smith *et al*., 2012). However, amongst the discoveries of altered brain structure and function, reduction in volumes of the prefrontal cortex and hippocampus was observed to be most consistent. This volume reduction is inversely correlated with length of illness, time of treatment and severity of depression (MacQueen and Frodl, 2011). The deleterious effects of stress could contribute to the reduced volume in these brain regions in depressed patients (Ridout *et al*., 2016). Conversely, the actions of antidepressant treatment could be mediated in part by blocking or reversing the atrophy caused by stress and depression. Some studies have identified a novel rapid acting antidepressant ketamine in treatment of resistant patients that addresses the limitations of currently available agents (delayed onset of action and low response rates) (Miaomiao *et al*., 2017). Ketamine, an N-methyl

d-aspartate (NMDA) receptor antagonist causes a rapid induction of synaptogenesis and spine formation in the prefrontal cortex via the stimulation of the mammalian target of rapamycin (mTOR) signaling pathway and increased synthesis of synaptic proteins. These effects of ketamine rapidly reverse the atrophy of prefrontal cortex neurons caused by chronic stress and correspond to rapid behavioural actions of ketamine in models of depression (Duman and Li, 2012). Moreover, the blockage of mTOR signaling pathway completely blocked ketamine induction of synaptogenesis and behavioural responses in models of depression. This justify that the effects of ketamine are opposite to the synaptic deficits that result from exposure to stress (Liu *et al*., 2010). So also, clinical studies reported that scopolamine (an acetylcholine muscarinic receptor antagonist) produces rapid antidepressant effects in depressed patients following a single low dose by rapidly increasing mammalian target of rapamycin c1 (mTORC1) signaling, and the number and function of spine synapses in layer V pyramidal neurons in the prefrontal cortex (Voleti *et al*., 2013).

Glutamate as a major excitatory neurotransmitter in the brain has also been implicated in depression (Zarate *et al*., 2006). Release of glutamate trigger the depolarization of postsynaptic neurons. Amino methyl-4-isoxazole propionic acid (AMPA) and NMDA receptors are two major ionotropic receptors that are especially suspected or being involved in learning and memory (Gerhard *et al*., 2016). While AMPA receptor activation leads to depolarization via sodium influx, NMDA receptor activation leads to depolarization via calcium and sodium influx. The calcium influx triggered through NMDA receptors can lead to activity dependent expression of many different genes, proteins and receptors that are involved in processes involving learning, memory, neurogenesis and environmental responses. The activity dependent synaptic responses also lead to rapid insertion of AMPA receptors into the post synaptic membrane which

act to maintain ongoing glutamatergic transmission as sustained calcium influx could result in activation of several signal transduction pathways and can stimulate the release of co-operative proteins such as BDNF. In long term potentiation signaling from NMDA, neuromodulators and growth factors converge to promote synaptic AMPA trafficking, mTOR dependent protein translation and cyclic AMP response element binding (CREB) dependent gene transcription. In contrast, during long term depression, intracellular phosphatase signaling oppositionally regulates these pathways. Extrasynaptic NMDA represent a major mediator of negative neuroplastic signaling, associated pathways lead to CREB shut off, disruption of mitochondrial function and potentially cell death (Marsden, 2011).

Depression has been thought to be related to circadian rhythms (Jonathan *et al*., 2009). In addition to that, symptoms of sleep disruptions and polysomnographic changes indicate the relationship with circadian rhythm (Armitage, 2007). The circadian theories for major depressive disorder (MDD) proposed that endogenous pacemaker is misaligned with respect to sleep timing. Misalignment between timing of the clock and the timing of sleep result in depression especially in susceptible individuals (Lewy *et al*., 2006). Circadian rhythms of many biological variables such as the daily profiles of body temperature, cortisol, thyrotropin, prolactin, growth hormone, melatonin and excretion of various metabolites are disrupted in depressed patients. Although no clock gene polymorphism is apparently associated with major depression but polymorphism has been linked to the recurrence of bipolar depression. Most antidepressant drugs and mood stabilizers influence endogenous rhythms. For instance, the antidepressant agomelatine (a melatonin receptor agonist and a $5HT_{2C}$ receptor antagonist) improves disturbed sleep-wake rhythms in depressed patients and adjusts the circadian system by resynchronizing the body temperature and cortisol levels. Moreover, the selective serotonin reuptake inhibitor

(SSRI) fluoxetine appears to modulate clock gene. So also, chronotherapeutics such as sleep deprivation is shown to be efficient in clinical treatment of depressed patients. All these mentioned are evidences which clearly linked depression to circadian system dysregulations.

There has also been accumulating evidence indicating the reciprocal communication pathways between nervous, endocrine and immune systems (Olga *et al*., 2005). Epidemiological data have provided strong support that stress plays a great role in the aetiology of depression (Caspi and Moffitt, 2006; Verduijn *et al*., 2015) and it occurs in interaction with genetic factors (Kendler *et al*., 1995). The development of depressive symptoms mediated by stress involves several systems including the monoamines and inflammation factors interacting with the HPA axis, which exert a central role in the regulation of the stress response (Fekadu *et al*., 2016). The activity of HPA axis is governed by the secretion of corticotrophin releasing hormone (CRH) and arginine vasopressin (AVP) from the hypothalamus which in turn activate the secretion of adrenocorticotropic hormone (ACTH) from the pituitary. ACTH then stimulates the secretion of corticoids (cortisol in humans and corticosterone in rodents) from the adrenal cortex. These hormones interact with their receptors (low affinity glucocorticoid receptors- GRs and high affinity mineralocorticoid receptors- MRs) in multiple target tissues throughout the body including the brain. Glucocorticoids exert a negative feedback control on CRH and AVP secretion through the activation of GRs expressed among others by hippocampal and paraventricular neurons. Besides the involvement of the receptors in negative feedback regulation, they also enhance hippocampal function and thereby promote certain cognitive abilities (James *et al*., 2016). The corticosteroid receptor hypothesis of depression is based on dysfunctions of HPA axis observed in the majority of depressed patients (Anacker *et al*., 2011; Pariante, 2003). HPA hyperactivity

results from the negative feedback regulation of the axis as evidenced by the failure of GR activation to decrease plasma levels of cortisol in the dexamethasone suppression test (Fountolakis *et al*., 2008). In this test, a low dose of exogenous steroid dexamethasone activates GRs and suppresses cortisol secretion in healthy individuals. A high proportion of depressed patients are non-suppressor in the dexamethasone test particularly individuals with severe psychotic symptoms and suicidal ideation (Zupanc *et al*., 2013). So also, dexamethasone administration suppresses mitogen induced lymphocyte proliferation and the production of the pro-inflammatory cytokine interleukin 1β in healthy controls but not in depressed patients (Vogel *et al*., 2012). Dexamethasone administration also induces an increase in the number of neutrophils and a decrease in the number of lymphocytes in healthy controls, and these effects are not observed in non-suppressors, indicating that depression is also associated with changes in immune function which confer a resistance to the effects of glucocorticoids on immunity. Cytokine mediated inflammatory processes might play an important role in neurochemical changes associated with depression (Audet and Anisman, 2013). Both glucocorticoids and pro-inflammatory cytokines lead to a decrease in the synthesis of 5-HT and increase neurotoxic metabolites by enhancing the conversion of its precursor tryptophan into kynurenine (Maes *et al*., 2011). This occurs via the induction of the enzymes indoleamine 2, 3-dioxygenase (IDO) by pro-inflammatory cytokines and tryptophan 2,3-dioxygenase (TDO) by glucocorticoids (Maes *et al*., 2012), which both convert tryptophan into kynurenine (inactive) which is degraded further to other forms of metabolites depending on cell type where they are produced or transported to, example, in microglia, kynurenine is degraded to 3-hydroxykynurenine and quinolinic acid (neurotoxic) (Baran *et al*., 2010). Thus, result in decreased 5-HT synthesis via tryptophan hydroxylase 2 (TPH2) (Pae and Wang, 2016). Depressive disorders are highly prevalent

in infectious, autoimmune and neurodegenerative disorders (Pollak and Yimirya, 2002) and in turn show high levels of pro-inflammatory cytokines and other proteins involved in immune response (Raison *et al*., 2009). Interestingly, half of the patients treated with cytokine interferon α develop depressive symptoms that are treated with classical antidepressants (Raison *et al*., 2006), indicating further how inflammation factors trigger depression. Furthermore, antidepressants were shown to reduce inflammation, possibly through the release of pro-inflammatory cytokines from activated macrophages, via monoaminergic receptors located on immune cells (Leonard, 2010).

At the anatomo-functional level, the hippocampus is observed highly sensitive to the deleterious effects of stress. Neuronal loss has been reported severally in the hippocampus of stressed or corticosterone treated animals. Moreover, human brain imaging studies illustrate how stress and depression may cause reduction of the hippocampal volume, probably because of dendritic aborization atrophy and loss of neurons, as observed in post mortem hippocampi of patients suffering from major depressive disorder (Stockmeier *et al*., 2004, Zhang *et al*., 2014). These morphometric changes are often attenuated or even reversed by antidepressants, suggesting the fact that they are related to depression. Furthermore, stress and glucocorticoids exert a drastic negative effect on the rate of cell proliferation, leading to a rapid and prolonged decrease in neurogenesis (Zimmerman *et al*., 2016). Regardless of the nature of hippocampal damages, these changes are likely to reduce the inhibitory control that the hippocampus exerts on the HPA axis, which would increase circulating glucocorticoid levels and lead to subsequent hippocampal dysfunction. Although the ablation of neurogenesis has no effect on basal HPA axis activity, it prevents monoaminergic antidepressant drugs from restoring the negative inhibitory function of the hippocampus when glucocorticoid levels become elevated in chronic stress conditions (Surget *et al*., 2011). Interestingly, recent

findings support the hypothesis that hyperactivity of the HPA axis is not a simple consequence or an epiphenomenon of depression but a risk factor predisposing the patient to development of depression (Parinte and Lightman, 2008) brought about by genetic liability as well as by early life experiences which programme molecular changes including epigenetic modifications (Meaney *et al*., 2007).

Epigenetics factors independent of DNA sequence variations is considerable in understanding the effect of early life stress in depression. Despite the fact that epigenetic programme is ruled by a highly organized developmental process tightly controlled to maintain tissue specific patterns of gene expression and is generally similar in different individuals, it is suggested that it might still be sensitive to input from the environment, especially in early life period (Tsankova *et al*., 2007). Environmental conditions can modulate the pattern of DNA methylation, while maintaining the overall cell-type specificity. Cues from social and physical environments early in life can cause variations in epigenetic programming to serve as an adaptive response of the genome to the anticipated lifelong environment (Szyf, 2009). Any misfit between the adaptive response and the actual environment later in life would result in maladaptation and an increased risk of developing diseases. As regard to this context, certain environmental exposures early in life might result in persistent epigenetic reprogramming that contributes to the risk of developing psychiatric diseases (Zhang and Meaney, 2010). For example, the role of DNA methylation variations in sustaining the effects of early environmental experience in mood related disorders, notably the dysregulation of the HPA axis activity linked to the postnatal mother-infant interactions (Weaver *et al*., 2004). This is reported to involve 5-HT-dependent synaptic transmission and binding of the nerve growth factor-induced clone A (NGFIA) transcription factor to the glucorticoid receptor (GR) promoter site. Others include the BDNF or arginine/vasopressin modifications linked to depression

(Tsankova *et al*., 2006; Sirianni *et al*., 2010). Thus, various classical antidepressants compounds were reported to exhibit epigenetic effects whereas histone deacetylases or DNA methylation inhibitors exhibit antidepressant like effects (Boks *et al*., 2012).

2.3 Aetiology

Depression is not a simple condition; thus the causes of depression remains unclear. There are several possible causes of depression that are diathesis-stress models in which depression is triggered in those vulnerable to it (Marsden, 2013). Depression may be an inherited condition where a person has a higher likelihood of experiencing it at a point in his or her life if they have a family member with depression (Duric *et al*., 2017). The possible causes of depression are categorized into the followings:

2.3.1 Biochemical

Some people have noticeable changes on their brains with depression. Neurotransmitters in the brain (serotonin, dopamine and norephinephrine) may be out of balance and result in depressive episodes (affecting feelings of happiness and pleasure) (Krishnan and Nestler, 2010).

2.3.2 Hormonal

Alterations in hormonal production and function lead to the onset of depressive states. Many changes in hormonal states, as in menopause, childbirth and thyroid disorder results in depression. For example, in postpartum depression, mothers develop symptoms of depression after child birth. This has been reported to be a serious condition. Scientists reported that certain hormones made deep in the brain trigger attitude related changes at certain times of the year (Hage and Azar, 2012).

2..3.3 Seasonal

This occurs as the daylight reduces in the winter. This results in people developing feelings of lethargy, tiredness and loss of interest in daily tasks (Meyerhoff *et al*., 2018).

2.3.4 Stimulation

This is linked to traumatic times, changes or struggle in life triggering depression. Losing a loved one, being fined, having financial troubles or undergoing a serious change can have big impact on people. For example, post-traumatic stress disorder (PTSD) is a form of depression that occurs after a serious situation in life (Aiping *et al*., 2016).

2.4 Types of Depression

There are several types of depression, some are associated with changes in life events while others are linked to chemical changes in the brain.

2.4.1 Major depression

This is the type of depression where a person feels depressed most of the time for most days of the week. Major depression is associated with symptoms such as loss of interest or pleasure in your activities, weight loss or gain, sleep, being tired and without energy, feeling worthless or having suicidal thoughts. A person suffers major depression if he has five or more of these symptoms on most days for 2 weeks (Conolly and Thase, 2016).

2.4.2 Persistent depressive disorder

A form of depression that lasts for two years or longer. It is called dysthymia. It is associated with symptoms such as not eating or overeating, sleeping too much or too little, lack of energy or fatigue, low self-esteem, trouble concentrating or making decisions, feeling hopeless etc. It is mild and yet enduring type of major depression. People with

dysthymia may appear to be chronically mildly depressed to the point that it seems to be part of their personality (Stynen *et al*., 2015).

2.4.3 Bipolar disorder

This is also called manic depression. It has mood episodes that range from extreme of high energy with an up mood to low depressive periods. When in the low phase, the symptoms are that of major depression (Tristchler *et al*., 2014).

2.4.4 Seasonal affective disorder

It is also called seasonal depression. It is a kind of mood disorder that happens every year at the same time. There is also a rare form of seasonal depression called summer depression. It begins in late spring or early summer and ends in fall (Benazzi, 2006).

2.4.5 Psychotic depression

This form of depression is associated with both symptoms of major depression along with psychotic symptoms such as hallucinations, delusions and paranoia (Schartzberg, 2003).

2.4.6 Post partum depression

This depression occurs in women weeks and months after childbirth (Chaudron, 2003).

2.4.7 Premenstrual dysphoric disorder (PMDD)

This depression occurs in women at the start of their period. Mood swings, irritability, anxiety, poor concentration, fatigue, change in appetite, changes in sleep habit and feeling of being overwhelmed are symptoms associated with PMDD in additions to feeling depressed (Thapar *et al*., 2012).

2.4.8 Situational depression

This type of depression occurs in individuals having difficulty in managing stressful event such as death of loved ones, divorce and job loss. It is also called a stress response syndrome (Chopra, 2009).

2.4.9 Atypical depression

This depression is different from the persistent sadness of typical depression. In this case, a positive event can temporarily improve mood. It includes other symptoms like increased appetite, sleeping more than usual, feeling of heaviness in your arms and legs and oversensitive to criticism (Jacobsen *et al*., 2012).

2.4.10 Substance induced mood disorder

Depression may be caused or precipitated by a known or unknown physical medical condition such as hypothyroidism (Hage and Azar, 2012).

2.5 Epidemiology

Depression is a major cause of morbidity worldwide (Kesler and Bromet, 2013). Lifetime prevalence varies widely from 3% in Japan to 17% in the U.S. In most countries, the number of people who would suffer from depression during their lives falls within 8- 12% range (Kessler *et al*., 2005).

Population studies have consistently shown depression to be twice as common in women as in men (Andrade and Caraveo, 2003). Epidemiological data reviewed on the prevalence, course, sociodemographic and societal cost depicted major depression as a commonly occurring disorder (Kessler and Bromet, 2013). A number of social

demographic information correlates of major depression are found consistently across countries.

Depression is a significant contributor to the global burden of disease and affects 350 million people in all communities across the world (Ferrari *et al*., 2013). The world mental health survey conducted in 17 countries found that on average about 1 in 20 people is reported to have an episode of depression in the previous year (Follmer *et al*., 2017). Depressive disorders often start at a young age and reduce persons functioning, and are often recurring. Depression is the leading cause of disability worldwide (WHO, 2017).

In developing countries like Nigeria, research showed that maternal depression may be a risk factor for poor growth in young children. The maternal depression has a substantial influence on growth during childhood. Thus, the effects of maternal depression will affect not only this generation but also next (Rahman *et al*., 2008; Chopra, 2009).

2.6 Drug Treatment of Depression and Overview of Antidepressant Drugs

Depression is a disorder that affects the brain; thus medications that modify brain functions offer hope. Antidepressants in particular are used to alleviate symptoms of depression. The followings are types of antidepressants:

2.6.1 Selective serotonin reuptake inhibitors (SSRIs)

These are medications that counteract depression symptoms by decreasing serotonin blockers in the brain (Citrome, 2014). They are the most commonly prescribed class of antidepressants. Examples include sertraline, fluoxetine, citalopram, escitalopram, paroxetine, fluvoxamine and trazodone. SSRIs ease depression by affecting naturally occurring chemicals messenger (neurotransmitters) that communicate between brain

cells. SSRIs act to block the reabsorption (reuptake) of the neurotransmitter serotonin in the brain (Pae and Wang, 2016). Altering the balance of serotonin helps brain cells send and receive chemical messages, and in turn boost mood. In addition to antidepressants and anxiolytic effects, all SSRIs work in almost a similar way to cause similar side effects. These side effects include nausea, nervousness, agitation, restlessness, dizziness, reduced sexual desire or difficultly to reach orgasm or inability to maintain erection, drowsiness, insomnia, weight gain or loss, headache, dry mouth, vomiting and diarrhea (Wang *et al*., 2015).

2.6.2 Serotonin and norepinephrine reuptake inhibitors (SNRIs)

These relief depression symptoms such as irritability and sadness. They are also used to treat anxiety and nerve pain. SNRIs act to block the absorption (reuptake)of the neurotransmitters serotonin and norepinephrine in the brain, and help boost mood (Zimmerman *et al*., 2016). Examples include duloxetine, venlafaxine and desvenlafaxine. SNRIs are associated with similar side effect because of their similarities in mechanisms of action. However, because of differences in chemical composition, their effect may differ. The most common side effects include nausea, dry mouth, dizziness and excessive sweating. Other side effects include tiredness, difficulty urinating, agitation or anxiety, constipation, insomnia, headache, loss of appetite and sexual problems (Citrome, 2014).

2.6.3 Tricyclic antidepressants (TCAs)

Tricyclic antidepressants are often prescribed when SSRIs or other antidepressants failed. They are among the earliest antidepressants developed. TCAs are very effective but have been replaced with classes of antidepressants with fewer side effects (Fekadu *et al*., 2016). TCAs block the absorption of the neurotransmitter serotonin and norepinephrine to make these chemicals available in the brain. TCAs work by changing the level of one or more

neurotransmitters, thus affect other chemical messengers which can lead to a number of side effects (Liebelt, 2015). Examples include amitriptyline, amoxapine, desipramine, doxepin, imipramine, nortriptyline, protriptyline, trimipramine and maprolitine (a tetracyclic antidepressant). Some of these medications are available as liquids (oral solutions). Tricyclic antidepressants are used in other conditions like anxiety disorders and neurophathic pain (Vogel *et al*., 2012).

The most common side effects of TCAs and other tetracyclic antidepressants are dry mouth, drowsiness, increased appetite leading to weight gain, light headedness and increased sweating. Others include disorientation and confusion (in the elderly on high dose), tremor, increased or irregular heart rate, frequent seizures in patients with seizures, difficulty in erection, delayed orgasm and low sex drive (Baghai *et al*., 2008).

2.6.4 Dopamine reuptake blockers

This class of antidepressant is a mild dopamine and norepinephrine reuptake blocker used for depression, seasonal affective disorder (SAD) and smoking cessation. It is represented by only one drug bupropion. This drug is associated with minor side effect such as nausea, vomiting, dry mouth, headache, constipation, increased sweating, joint aches, sore throat, blurred vision, strange taste in the mouth, and dizziness. Although serious side effects are unlikely but they do occur. They include chest pain, fainting, fast irregular heartbeat, hearing problems, ringing in the ears, severe headache, mental or mood changes (such as anxiety, agitations, hallucinations, memory loss, confusion), tremors, unusual weight gain or loss, muscle pain, change in the amount of urine, vision changes, seizures, allergic reaction (rashes, itching, swelling), severe dizziness and trouble breathing among others (Sagud *et al*., 2011).

2.6.5 5-HT_2 Receptor Antagonists

There are two drugs used to treat depression from this class, nefazodone and trazodone. Trazodone is approved and marketed in several countries worldwide for the treatment of major depressive disorder (MDD) and has demonstrated comparable antidepressant activity comparable to drug from classes like SSRIs, TCAs, and SNRIs (Fagiolini *et al*., 2012). The exact mechanism of action is not known, but is likely related to enhancing serotonergic activity in the CNS. It inhibits serotonin transporter and is a 5-HT_{2A} and 5-HT_{2C} antagonist. It is antagonist at both histamine (H_1) and α_1 adrenergic receptors (associated with its sedating and hypotensive effects respectively). The most common adverse effects reported with this class are drowsiness (somnolence or sedation), headache, dizziness and dry mouth. Others include orthostatic hypotension (in elderly patients or those patients with heart diseases), minimal anticholinergic activity, corrected QT interval prolongation and torsade de pointes, cardiac arrhythmias, priapism and suicidal ideation.

2.6.6 5-HT_3 receptor antagonists

Votioxetine is the only 5-HT_3 receptor antagonist used in the treatment of depression. It is a novel drug that effectively treats symptoms of depression and cognitive dysfunction in adult (James *et al*., 2016). It differs from SSRIs in its multinodal effect on serotonin transport and reuptake. It also affects a variety of other neurotransmitters and it is the first antidepressant to affect the domain of cognition independently. The exact mechanism of its cognitive effect is not known but it may be related to its antagonistic at 5-HT_3 and 5-HT_7 receptors; where it may impact neurotransmitters like glutamine and acetylcholine that are intimately involved in regulation of cognitive function (Conolly and Thase, 2016). The most commonly reported negative side effects are headache and nausea. Other common adverse events include dizziness, dry mouth and diarrhea (Citrome, 2014).

2.6.7 Monoamine Oxidase inhibitors (MAOIs)

They are older antidepressants (first generation). MAOIs have more side effects than the newer (second generation) antidepressants. They act to balance brain chemicals (neurotransmitters) by reducing the amount of monoamine oxidase (the substance that breaks down the neurotransmitters) resulting in alleviating symptoms of depression (Polnak *et al*., 2018). MAOIs are used in patients with depression and are not doing well on other antidepressants, those that cannot tolerate side effects of the antidepressants, those who have a family or personal history of successful treatment with MAOIs and in those with unusual symptoms of depression such as weight gain and hypersomnia. MAOIs should not be the first drugs in depression because of their serious side effects when combined with certain foods and other drugs. It is not recommended for children or teens. Examples of MAOIs are isocarboxazid, phenelzine, selegiline (a transdermal patch) and tranylcypromine (Zabegalov *et al*., 2018).

Major side effects associated with MAOIs includes hives, chest pains, fast or slow heartbeat, severe headache, stiff neck, nausea or vomiting, trouble breathing, swelling of face, lips, tongue, or throat. These require prompt discontinuation of treatment (Tsutsui *et al*., 2018).

Common side effects of MAOIs include dizziness, (light headedness), high blood pressure, appetite changes or weight gain, loss of sexual desire and muscle twitching during sleep. However, serious reactions or even death can result when MAOIs are combined with some foods like fava beans, red wine as well as with other drugs like TCAs, cold remedies and diet pills. Eating these foods can cause severe high blood pressure and other health problems. Thus, a washout period of at least 14 days is required when MAOI is stopped before taking another antidepressant (Polnak *et al*., 2018).

2.6.8 Noradrenergic antagonists

An example of drug from this class is mirtazapine. It is a unique antidepressant that refines the specificity of effects of noradrenergic and serotonergic systems. It is an antagonist of α_2-adrenergic autoreceptors and heteroreceptors on both norepinephrine and serotonin (5-HT) presynaptic axons, and a potent antagonist of postsynaptic 5-HT_2 and 5-HT_3 receptors (Molendijik *et al*., 2011). The net outcome of these effects is increased noradrenergic activity together with specific increased serotonergic activity, especially at $5HT_{1A}$ receptors. This mechanism of action maintains equivalent antidepressant efficacy but minimizes many of the adverse effects common to both tricyclic antidepressants and SSRIs. Interestingly, mirtazapine has minimal cardiovascular and anticholinergic effects (Stimmel *et al*., 1997) and essentially lacks serotonergic effects such as gastrointestinal symptoms, insomnia and sexual dysfunction. The most common side effects associated with mirtazapine are sedation, increased appetite and weight gain.

2.6.9 Atypical antidepressants

They are used in patients with major depression with inadequate responses or intolerable side effects during first line treatment with SSRIs. However, atypical antidepressants are often first-line treatment if the drug has a desirable characteristic (e.g reduced sexual side effects and weight gain). An example of drug in this class is agomelatine, which is used to treat depression and other illnesses (Girish *et al*., 2010). It acts on brain receptors for melatonin, a hormone that is important in regulating sleep, as well as serotonin receptors. Agomelatine is the only antidepressant that acts on melatonin receptors (MT_3). Agomelatine has been observed to cause abnormal increase in liver enzymes. The common side effects of Agomelatine are dizziness, abdominal changes in liver function tests and abdominal pain. Other infrequent side effects are nausea, diarrhea, anxiety,

blurred vision, sleepiness, fatigue, excessive sweating and rash. Hepatitis is a rare side effect that has also been linked to Agomelatine treatment.

2.7 Plants used in the Management of Depression

As a therapeutic alternative, effective herbal drugs were found to offer advantages in terms of safety and tolerability, possibly also improving patients' compliance (Kamalipour *et al*., 2008). Plants extracts are some of the most attractive sources of new drugs and have been showing promising results for the treatment of depression. Examples of these plants include:

2.7.1 *Hypericum perforatum* L. (St. Johns Wort)

This is one of the best studied plant used in the treatment of depression. It is notable for its ability to treat mild to moderate depression. It is also known to be safe and effective for children. St. Johns Wort (SJW) is very popular in U.S and it is available over the counter. The possible activities of SJW have been linked to its hypericin and hypericin-like constituents, which is suspected to act on acetylcholinesterase by decreasing the degradation rate of acetylcholine. Sedative activity of SJW has been linked to hypericins, biflavones and hyperforin constituents. There is also a report on its serotonergic activity which account for its fewer side effects. Other studies showed effect of SJW on sigma l receptors. Most likely, the demonstrated efficacy of this plant is through its synergistic effect, orchestrated by the multitude of components in the whole herb working both within and peripheral to the central nervous system (Gaster and Holroyd, 2000). The action of SJW has been well characterized in direct comparisons with leading antidepressant medications to show better efficacy and safety profiles (Hansgen *et al*., 1994; Linde *et al*., 1996; Liebermann, 1998; Gelenberg, 2000; Kim *et al*., 2003; Kamalipour *et al*., 2008).

2.7.2 *Lavandula angustifolia* Mill (Lavender)

This is used as an aromatic essential oil for relaxation. A single blind randomnized control trial showed improved mood, reduced aggression and a more positive outlook in women who took daily baths with lavender oil (Abdullaev, 2002; Akhondzadeh *et al*., 2003). More so, a combination of lavender oil tincture (60 drops per day) and imipramine (100mg per day) was found to be more effective in the treatment of depression than either agent alone in a double blind randomized control trial. The findings of this study suggested that taking a moderate amount of lavender may help reduce the amount of antidepressant needed to treat depression, leading to fewer side effects.

2.7.3 *Crocus sativus* L. (Saffron)

The pharmacological action of this plant has been linked to three main secondary metabolites. These are crocin and its derivatives which are responsible for color, picrocrocin responsible for taste and safranal responsible for odor. Therapeutically, saffron is considered as an excellent aid for stomach ailments and antispasmodic, it helps digestion and increases appetite. It relieves renal colic, tension and stomach ache (Rios *et al*., 1996; Abe and Saito, 2000). It is used for depression in Persian traditional medicine. Clinical findings showed safety and efficacy of saffron in depression. A randomized, double –blind study showed that saffron extract 30 mg (capsules) given for 6 weeks resulted in significant alleviation of depression compared to placebo groups, without evident side effects. In other studies, saffron showed similar effects to fluoxetine and imipramine in the treatment of depression (Hosseinzadeh and Younesi, 2002; Farahani *et al*., 2015).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Drugs and Chemicals

The followings are some of the chemicals used for the experiment.

Imipramine (Tofranil GSK brand USA), Diazepam (Roche, France), Methanol (Fluka-Aldrich), Cortisol Enzyme-linked immunosorbent assay (ELISA) kit (Wuhan Fine Biotech Co. Ltd, Catalogue No. EM1721), Brain derived neurotrophic factor enzyme-linked immunosorbent assay kit (ELISA) kit (Wuhan Fine Biotech Co., Ltd., Catalogue No. EM0020).

3.1.2 Equipment and other Materials

Animal cages, pestle and mortar, syringes (1 ml, 2 mls, 5 mls and 10 mls), filter paper, pair of scissors, mettler balance p165, measuring cylinders, separating funnel, beakers, test tubes, funnel, test tube holders, crucible, water drinkers, Incubator (model: DHP-9035A), Spectrophotometer (model: Spectrumlab 23A) and microplate reader (Rayto-RT-2100C).

3.1.3 Animals

Swiss Albino mice (18-22 g) of either sex were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria. They were housed in improvised propylene cages and kept under natural day and light cycle. The animals were fed on standard laboratory animal diet and water *ad libitum*. All experimental protocols were as approved by the University Animal ethics committee with approval number ABUCAUC/2017/022 (Appendix 2).

3.2 Methods

The research was conducted in three phases.

Phase 1: Ethnobotanical survey, plants collections and extractions, then phytochemical screening.

Phase 2: Acute and neurotoxicity studies, followed by antidepressant studies which validated the folkloric claim of the surveyed plants.

Phase 3: The most active plants was selected for further depressive and mechanistic studies.

3.2.1 The Procedure for the Ethnobotanical Survey

An ethnobotanical survey was conducted in order to obtain relevant information about medicinal plants used in the treatment of depression in Zaria, a city in Kaduna state Northwestern Nigeria. Data was collected based on oral interview with the aid of semi structured questionnaire. Only data from willing respondents were obtained and documented. Plant specimens were collected along the line, subsequently dried and mounted. They were then taken for identification and authentication in the Herbarium Section of Botany Department, Ahmadu Bello University Zaria (Burkill, 1985; Sofowora, 2000).

3.2.1.1 Study area

The survey was carried out in Zaria, Nigeria. The occupation, tribe and population of inhabitant of this area formed part of bases for selection.

3.2.1.2 Ethical approval

The purpose of the study was explained to the respondents and informed consent was obtained from each respondent (Appendix 1).

3.2.1.3 Inclusion Criteria

Well known and established traditional herb sellers, traditional medical practitioners and herbalists who were healthy and mentally stable, practicing their jobs at the time of the survey participated in the sudy.

3.2.1.4 Exclusion criteria

Traditional herb sellers, traditional medical practitioners and herbalists with poor patronage, mentally ill and not practicing their jobs at the time of the study.

3.2.1.5 Administration of questionnaire

The survey covered a period of one month. Ethno-medicinal information was obtained by consulting traditional medical practitioners, herb sellers and herbalists using semi-structured questionnaire and an oral interview. The questionnaire was divided into three sections.

Section 1 dealt with demographic information such as age, sex, religion, nationality, practice specification, duration of practice and educational background.

Section 2 consisted of professional experience on the treatment of depression and included questions like type of depression treated, frequency of treatment, use of herbal

therapy alone or otherwise, duration of treatment, accompanied side effects, accompanied verbal instructions, plant part(s) use, availability of plant or plant part(s) and knowledge of treatment.

Section 3 focused on plants and recipes used in the treatment of depression requested included questions like types of herbal preparation, arrangement of plant part(s) ingredient, traditional solvent of choice, traditional extraction methods or method of preparation and method of administration.

3.2.2 Plant Collection and Extraction

Nine of the ethnobotanically surveyed plants (*Adansonia digitata*, *Ficus platyphylla*, *Senna occidentalis*, *Tapinanthus dodeinofolius*, *Tapinanthus globiferus*, *Caralluma dalzielli*, *Combretum micranthum*, *Pancratium africanum* and *Olax manni*) were collected. The plants were taken to the Herbarium Section of the Department of Botany, Ahmadu Bello University Zaria for identification and authentication.

The plants were transported to the Department of Pharmacognosy and Drug Development where they were dried under shade with intermittent weighing until constant weight obtained. The plants were then size-reduced using mortar and pestle. The powdered materials were extracted with absolute methanol via soxhlet extraction. The extracts were concentrated under reduced pressure and temperature 45^{o} C. The extracts were finally stored in desiccators until needed for the work. Solutions of extracts were freshly prepared for each study using distilled water.

3.2.3 Procedure for Phytochemical Screening

The methanol extracts of the plants above were subjected to phytochemical screening as follows:

3.2.3.1 Test for carbohydrates

Molisch test: Extract (0.5 g) was dissolved in water (3 mls) in a test tube. Three drops of molisch reagent were added then followed by 5 drops of concentrated sulphuric acid along the test tube wall and observed. A purple to violet colour at interface observed indicated the presence of carbohydrate (Evans, 2009).

3.2.3.2 Test for flavonoids

Sodium hydroxide test (Evans, 2009): About 2 mls of filtered extract was dissolved in 2 mls of 10% aqueous sodium hydroxide solution, a yellow solution resulted to which dilute HCl was added to form colourless solution which indicated the presence of flavonoids.

3.2.3.3 Test for cardiac glycosides

Keller Killiani's test: Glacial acetic acid (1 ml) was added to about 3 mls of the solution which contained 2 mg of extract in a test tube held at 45^{o}; then two drops of concentrated H_2SO_4 was added along the side of the test tube. Formation of purple ring colour at the interface observed indicated the presence of cardiac glycosides (Evans, 2009).

3.2.3.4 Test for saponins

Extract (0.5 g) was shaken with 3 mls of water for 30 seconds and allowed to stand for 30 minutes. Formation of honey comb which persisted for more than 30 minutes when observed indicated the presence of saponins (Sofowora**,** 1993).

3.2.3.5 Test for tannins

Lead sub-acetate test: Extract (0.5g) was dissolved in 2 mls of water; 3 drops of lead sub-acetate solution were then added and observed for formation of black-green coloured precipitate indicated the presence of tannins.

3.2.3.6 Test for steroids and triterpenes

Liebermann-Burchard's test: Powdered plant material (0.5 g) was extracted with 5 mls of methanol then filtered. The filtrate was evaporated to dryness on a water bath at 100^{o} 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 1 ml of concentrated sulphuric acid was also added carefully down the zone of contact of the two liquids. A brownish-red colour was observed immediately at interface and violet blue-green at the upper layer later. Red colour indicates the presence of triterpenes while blue-green colour indicates steroids (Evans, 2009).

3.2.3.7 Test for alkaloids

Extract (0.5 g) was treated with 5 mls of 1% aqueous HCl and then heated. It was filtered and the filtrate divided into four portions in four test tubes **(**Sofowora, 1993).

a. Dragendoff's reagent: to test tube 1, two drops of dragendoff's reagent was added and rose red precipitate observed indicated presence of alkaloids.
b. Wagner's reagent: to test tube 2, two drops of Wagner's reagent was added and brownish precipitate observed indicated the presence of alkaloids.
c. Mayer's reagent: to test tube 3, two drops of Mayer's reagent was added and creamy precipitate observed indicated the presence of alkaloids.

3.2.4 Thin Layer Chromatographic Profiling

The extract was dissolved and various spots were formed on precoated silica gel PF_{254} (0.2 mm thick) thin layer chromatographic (TLC) plate with the aid of capillary tube in about 0.5 cm above the bottom edge and 0.5 cm away from the sides. The spots were allowed to dry, thne TLC plates were placed in a development chromatank containing the mobile phase prepared 30 minutes before placing plates. The mobile phase was allowed to run along the TLC plate in an ascending manner. When the mobile phase reached a desired distance, the plates were removed, the solvent front was marked and the plates were dried and visualized under ultra violet light (254 nm and 366 nm) for fluorescence. One plate was sprayed with a general reagent (p-anisaldehyde solution) and other plates with specific spraying agents such as Liebermann-Burchard reagent (for steroids and triterpenoids) or ferric chloride (for phenolic compounds), aluminium chloride (for alkaloids) and Dragendorff (for alkaloids). This was followed by heating at 110℃ for 5 minutes and observed for the presence or absence of various secondary metabolites (Haborne, 1991; Ghani, 1990).

3.2.5 Acute Toxicity Studies in Mice (LD_{50}): Up and Down Method

LD_{50} determination was conducted using Organization for Economic Co-operation and Development (OECD 425) guidelines in mice. In this method, two groups each of three animals were fasted prior to dosing (3 hours for mice). The fasted body weight was determined for each animal and dose calculated according to the body weight. Food was further withheld for 1-2 hours in mice after the methanol extract of *Adansonia digitata* had been administered. The extract was administered orally using an oral canula. A start dose of 5,000 mg/kg (limit test) was used for one mouse in the first phase. Animals dosed in the first phase were observed for 48 hours, the test proceeded to the second phase

depending on the outcomes of first phase. Additional two animals that survived for a particular extract were dosed and observed individually at least once during the first 30 minutes after dosing and periodically during the first 24 hours, with closer observation during first 4 hours and then daily for 14 days. Additionally, mice that died in the first phase from a particular extract, the second phase was conducted at dose of 2,000 mg/kg, were one mouse was dosed and observed for 24 hours. When no death was observed, four additional mice were also treated with 2000 mg/kg and were observed as in the first phase. Observations made included changes in skin and fur, eyes and mucous membranes, somator activity and behaviour pattern, autonomic and central nervous systems etc. Animals were observed for tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Time of onset of toxic symptoms and disappearance were also noted. Individual weights of animals was determined daily for two weeks.Thesame procedure was repeated for methanol extracts of *Ficus platyphylla*, *Senna occidentalis*, *Tapinanthus dodeinofolius*, *Tapinanthus globiferus*, *Caralluma dalzielli*, *Combretum micranthum*, *Pancratium africanum* and *Olax manni*.

3.2.6 Neurotoxicity Studies (Beam Walking Assay)

Mice were trained to walk from a start platform along a ruler (80 cm long, 3 cm wide) elevated 30 cm above the bench by a wooden support to a goal box. Three trials were performed for each mouse, and were designed such that the mice tested would be aware that there was a goal box that could be reached. A ruler was used because the mouse found this easy to cross, and at the same time, it induced minimum anxiety. The mice that successfully walked along the ruler were randomly grouped into five groups each containing eight mice. The first, second and third group received graded doses of extract orally one hour prior to test. The fourth and fifth groups received normal saline (10 ml/kg) and diazepam (10 mg/kg) orally respectively. One hour post-treatment, each mouse was

placed on the beam (60 cm long, 8 mm in diameter and 30 cm elevated above the bench) 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 beam. The measurements taken were time to complete the task, the number of foot slips (one or both hind limbs slipped from the beam) and the number of falls (Stanley *et al*., 2005). The same procedure was repeated for each plant extract.

3.2.7 Antidepressant Screening

3.2.7.1 Tail suspension test (TST)*:* Mice were transported to neurobehavioural laboratory and adapted for 1 hour. Forty mice were divided into five groups of eight animals each. Group 1, 2 and 3 were treated with the test extract (250, 500 and 1,000 mg/kg for LD_{50} ≥5000 mg/kg; 125, 250 and 500 mg/kg for LD_{50} ≥2,000 mg/kg) orally 1 hour prior to test. Group 4 and 5 were treated with distilled water (10 ml/kg) and imipramine (15 mg/kg) respectively. For the test, mice were suspended on the edge of the shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility was recorded for a period of 6 minutes (Steru *et al*., 1985). The same procedure was repeated for each extract.

3.2.7.2 Open field test (OFT)*:* Forty mice were divided into five groups of eight animals each. Group 1, 2 and 3 were treated with the test extracts (250, 500 and 1,000 mg/kg for LD_{50} ≥5,000 mg/kg; 125, 250 and 500 mg/kg for LD_{50} ≥2,000 mg/kg, *po*) 1 hour prior to test. Group 4 and 5 were treated with distilled water (10 ml/kg, *po*) and diazepam (10 mg/kg, *po*) respectively. Each mouse was placed in white wooden open field apparatus (70×70×35 cm, length×breadth×height) of which one wall is plexiglass. And also a

plexiglass floor divided into 16 visible squares (15×15 cm) with a central square. Behavior of mice such as peripheral and central square crossing was recorded for 5 min. Arena was cleaned with 10% ethanol between tests (Rex *et al*., 1998). The same procedure was repeated for each extract.

The most promising plant that showed specific antidepressant activity with no previous report on its antidepressant effect was *Adansonia digitata*. Thus, it was selected for further depressive and mechanistic studies.

3.2.8 Forced Swim Test (FST) Procedure

Forty mice were taken to neurobehavioural laboratory one day before the experiment and housed separately in cages with free access to food and water. They were divided into five groups of eight animals each. Group 1, 2 and 3 were treated with the methanol stem bark extract of *Adansonia digitata* (250, 500 and 1,000 mg/kg) orally. Group 4 and 5 were treated with distilled water (10 ml/kg, *po*) and imipramine (15 mg/kg, *po*) respectively. One hour after treatment, mice were individually forced to swim inside a vertical plexiglass cylinder (height: 24 cm, diameter: 12 cm, containing 12 cm of water maintained at 25 °C). The total duration of immobility is measured for 5 minutes. An animal is judged immobile whenever it remains floating passively in the water in a slightly hunched but upright position, its nose just above the surface (Alpermann *et al*., 1992).

3.2.9 Novel Object Recognition Test (NORT) Procedure

The test involved the use of an apparatus which consisted of black plastic boxes (28 × 41 × 18 cm) slightly filled with sawdust (≈0.5–1 cm thickness) in a room with a low level of light. Locomotor activity was observed during the entire experiment (parameter: ambulatory distance) using a videotracking procedure. Objects exploration was hand-scored. The NORT was divided into 2 training sessions and one test session. Each exposure lasted for 5 min with a 3-min inter-trial interval. Between each trial, mice were returned to their home cage, bedding of apparatus was changed and boxes cleaned with 70% ethanol solution. During training sessions, two identical objects; cylindrical glassware (diameter: 3 cm, height: 8 cm) filled with white cotton and placed in the box. Each mouse was placed in the middle of the box facing the wall and was allowed to freely explore the apparatus and the objects. During the test session, one of the familiar objects was removed from the cage and replaced by a novel object rectangular structure (7 × 3 × 9 cm). The objects were previously validated to ensure that no inherent preference for either object. The nature (Novel vs familiar) and the position of the novel object (left vs. right) were chosen randomly (Sahay *et al*., 2011).

Object exploration is defined as the orientation of the nose to the object at a distance ≤ 2 cm. Placing the forepaws on the objects was considered as exploratory behavior, but climbing on the objects was not. Objects was cleaned with 70% ethanol between trials to avoid olfactory cues. Results for this test was expressed as: (1) exploration of each object (in seconds) during test sessions (2) a discrimination index (DI) between objects during the test session, calculated as the difference between the time spent exploring the novel object (N) and the familiar object (F) divided by the total time exploring both objects

$$(D1) \quad = \frac{(N-F)}{(N+F)}$$

3.2.10 Chronic Unpredictable Mild Stress (CUMS) Procedure

The chronic unpredictable stress model was induced by chronic variable stress with modification (Murua *et al*., 1991). Based on consummatory behaviour, mice were matched and assigned to stress and control groups. Stressed mice were then singly housed and exposed to the following stressors: 2-hours immobilization, 24-hours food deprivation, 24-hours water deprivation, 5-min cold swim at 4^0C, wet bedding, 24-hours cage tilt at 45 degrees, 24-hours empty cage exposure, 24-hours cage reduction and 1-minute tail pinch with clothes needle placed 1 cm distal from the base of tail. The same stress was not applied successively so that mice do not anticipate the occurring of stress. At the end of stress session, mice were returned to their home cages with free access to food and water except for the 21-hours period of food and water deprivation before the sucrose consumption test until next stress session. Also in the case of non-stressor group, mice were kept in one cage with access to water and food except for 21-hours deprivation prior to sucrose consumption test. They were not disturbed except for the purpose of weighing and cage cleaning.

3.2.10.1 Experimental procedure: Seventy-two mice were randomly divided into six groups matched for weight and sucrose consumption. Group 1 mice were normal control (no stress and no drug), group 2 mice were stressed group (stressed+ distilled water, *po*), group 3 mice received stressed+ MEAD 250 mg/kg, group 4 mice received stressed+ MEAD 500 mg/kg, group 5 mice received stressed+ MEAD 1,000 mg/kg and group 6 mice received stress+ Imipramine 15 mg/kg. Mice were treated orally and assessment was done one hour after drug administration. The stress procedure lasted for 5 weeks. Drug were administered for three weeks, starting two weeks after stress exposure up to the end of stress procedure. Open field test, tail suspension test and sucrose preference

test were carried out thrice each (before stress, before drug administration and at the end of drug administration). Mice were sacrificed after the last open field test. The whole experiment lasted for 45 days in the following temporal sequence: (1) Animal adaptation: 1-3 days; (2) open field test: 4, 18, 39 days; (2) sucrose consumption test: 5-8, 22, 43 days; (3) tail suspension test: 9, 23, 44; (4) stress procedure: 10- 45 days; (5) drug administration: 24- 45 days.

3.2.10.2 Consummatory Behaviour: extensive preliminary tests for preference of cage side, sucrose preference over water, sucrose concentration and one- versus two-bottle paradigm conducted before the CUMS period. Liquid intake was determined by measuring the volume of sucrose consumption within 24-hours. Sweet solutions were offered on the preferred right cage side, as a shifting from the preferred solution at the preferred cage side to water at the other side might more closely model anhedonic behaviour (Matt and Shieh, 2015).

3.2.10.3 Sucrose preference test: Mice were trained to consume 2% ($^{w}/_{v}$) sucrose solution before the experiment. They were exposed to two bottles, one bottle of 2% sucrose solution in a 24-hour period and one bottle of drinking water for next 24-hours period. Formal test was carried out before stress, 2 and 5 weeks after stress. Mice were food and water deprived for 21-hours and they were exposed to both the test solution (2% sucrose) and drinking water within that period. Sucrose consumption was measured by measuring the volume of sucrose solution remaining in the test bottle and subtracting from the 20 mls initial volume added (Forbes *et al*., 1996).

3.2.10.4 Tail suspension test: The test was carried at 0, 2 and 5 weeks after stressors. For the test, mice were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1cm from the tip of the tail. The duration of immobility was recorded for a period of 6 minutes (Steru *et al*., 1985).

3.2.10.5 Open field test: The test was carried out before stress, 2 weeks after stress and 5 weeks after stress. Each mouse was placed in white wooden open field apparatus (70×70×35 cm, length×breadth×height) of which one wall is plexiglass. And also a plexiglass floor divided into 16 visible squares (15×15 cm) with a central square. Behavior of mice such as peripheral and central square crossing and rearing was recorded for 5 min. Arena was cleaned with 10% ethanol between tests (Rex *et al*., 1998).

3.2.10.6 Blood sampling collection and brain harvest: On day 46 of the experiment, mice were left without any drug administration. Eight mice were randomly selected from each of the six groups and sacrificed by decapitation. Blood samples were collected in plain bottles and centrifuged at 1000 X g at 8 ℃ within 30 minutes. Plasma and erythrocytes were then separated, used for cortisol competitive ELISA detection, SOD activity and MDA levels assessment. Additionally, brain samples were harvested and stored in 0.1 M sodium phosphate buffer, pH 7.4 at -20℃ till needed for further investigations. Brain was minced and homogenized in PBS buffer (0.01M, pH 7.4) with a glass homogeneizer on ice. Homogenates were centrifuged at 5000 X g in which supernatant was obtained. The brains homogenate was used for sandwich enzyme-linked immunosorbent assay.

3.2.10.7 Superoxide dismutase (SOD) activity assay: SOD activity was measured using calorimetric assay. SOD radical anion (O_2), the substrate of SOD was generated indirectly

in the oxidation of epinephrine at alkaline pH by the action of oxygen. The pink oxidation product of epinephrine (adrenochrome) was measured spectrophotometrically at 485nm (Misra and Fridovich, 1972). Briefly, to 875 µl of 50 mM carbonate buffer, pH 10.2, 25 µl of separated erythrocytes diluted 100 fold and 100 µl of 10 mM epinephrine were added. The absorbance at 585 nm was measured for 3 minutes. As a standard 25 µl of SOD solution with known activity was used.

3.2.10.8 Malonaldehyde (MDA) determination: The plasma MDA level was assayed using thiobarbituric acid spectrophotometric method with 1, 1, 3, 3 tetraethoxypropane as a standard (Ohkawa *et al*., 1979). TBA reacts with some products of lipid peroxidation in acidic environment at increased temperature to form a pink compound. Briefly, 250 µl plasma were added to 250 µl of distilled water, 500 µl of 15% TCA and 500 µl of 0.37% TBA. TCA and TBA solutions were prepared in 0.25 M HCl. The samples were heated in a boiling water for 10 minutes. After cooling, the samples were centrifuged at 12,000 X g. The absorbance was measured at 535nm.

3.2.10.9 Measurement brain-derived neurotrophic factor (BDNF) level: BDNF level was measured using a commercially available enzyme-linked immunosorbent assay kit (ELISA) kit according to the manufacturer's instructions. Briefly, the plate was washed twice before adding standard, sample and zero wells. Then 100 µl of standard, samples were added to each well and incubated for 90 minutes at 37°C, after which plates were aspirated and washed three times. Then 100 µl of Biotin labelled antibody working solution was added to each well and incubated for 60 minutes at 37°C, after which plate was removed, aspirated and washed three times. Then 100 µl of SABC Working Solution

was added into each well and incubated for 30 minutes at 37℃. Plates were removed, aspirated and washed five times, then followed by addition of 90 µl TMB Substrate and incubated lastly for 15 minutes at 37℃. Plates were removed and 50 µl of stop solution was added into each well. Absorbance was measured immediately at 450 nm using a microplate reader. The sensitivity of the assay was <2.0 pg/ml of BDNF.

3.2.10.9 Measurement of serum cortisol level: Serum cortisol level was measured using a commercially available enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol. Briefly, plates were washed twice before samples added, then 50 µl of sample and standard solutions were added to the already precoated antibody plate provided with the kit and incubated for 45 minutes at 37 ℃. Plates were removed, aspirated then washed three times, followed by addition of 100 µl of SABC Working Solution into each well, then incubated for further 30 minutes at 37 ℃. Plates was removed, aspirated and washed five times, followed by addition of 90 µl of TMB Substrate. Plates were further incubated for 15 minutes at 37℃. The reaction was stopped by adding 50 µl of stop solution and absorbance was read at 450 nm using a microplate reader (Rayto-RT-2100C). The sensitivity of the assay was <0.234ng/ml of cortisol.

3.2.11 Determination of the Possible Mechanism of Action of the Methanol Stem Bark Extract of *Adansonia digitata*

3.2.11.1 To determine the involvement of Dopaminergic Pathway

The possible involvement of dopaminergic pathway in the observed antidepressant activity of methanol stem bark extract of *A. digitata* was determined using tail suspension test (TST). Forty mice were divided into five groups each of 8 mice. The first, second and

third groups were administered distilled water (10 ml/kg), MEAD (1,000 mg/kg) and imipramine (15 mg/kg) orally respectively. Group 4 and 5 mice were pretreated with sulpiride (50 mg/kg, *ip*) a dopamine D_2/D_3 receptors antagonist 15 minutes prior to administration of MEAD (1,000 mg/kg) and imipramine (15 mg/kg) orally respectively. One hour later, mice in all groups were assessed using TST (Gay *et al*., 2010).

3.2.11.2 To assess the involvement of Serotonergic Pathway

Involvement of serotonergic system in the antidepressant activity of methanol extract of *A. digitata* was determined using TST in mice. Mice were divided into seven groups with eight mice in each group. Groups 1, 2 and 3 were treated with distilled water (10 ml/kg), MEAD (1,000 mg/kg) and imipramine (15 mg/kg) via oral route respectively. Groups 4 and 5 mice were pretreated with metergoline (1 mg/kg, *ip*) a serotonin 5-HT_1 receptor antagonist and groups 6 and 7 mice were pretreated with cyproheptadine (3 mg/kg, *ip*) a serotonin 5-HT_2 receptor antagonist. Fifteen minutes later, mice in group 4 and 6 were treated with MEAD (1,000 mg/kg), and group 5 and 7 were treated with imipramine (15 mg/kg) orally. One hour after treatment, mice in all groups were subjected to TST.

3.3.5.3 To investigate the possible involvement of Noradrenergic System

To investigate the possible involvement of the noradrenergic system in the antidepressant action of MEAD, 56 mice grouped into 7 groups of 8 mice each were used. The first, second and third groups received distilled water (10 ml/kg), MEAD (1,000mg/kg) and imipramine (15 mg/kg) orally respectively. The fourth and fifth groups were pretreated with prazosin (1 mg/kg, *ip*) an α_1 adrenergic antagonist 15 minutes prior to administration of MEAD (1,000 mg/kg) and imipramine (15 mg/kg) orally respectively. The sixth and seventh groups were pretreated with yohimbine (1 mg/kg, *ip*) an α_2 adrenergic antagonist,

15 minutes later, MEAD (1,000 mg/kg) and imipramine (15 mg/kg) were administered orally respectively. Mice in all the groups were assessed one hour later using TST.

3.3.5.4 To determine the possible involvement of the Nitric Oxide Pathway

Fifty-six (56) mice were grouped into 7 groups each of 8 mice. Groups 1, 2 and 3 were treated with distilled water (10 ml/kg), MEAD (1,000 mg/kg) and imipramine (15 mg/kg) orally respectively. Groups 4 and 5 were pretreated with L-Arginine (50 mg/kg, *ip*) a substrate of nitric oxide synthase and group 6 and 7 were pretreated with L-NNA (50 mg/kg, *ip*) a nitric oxide synthase enzyme inhibitor. Fifteen minutes later, groups 4 and 5 were treated with MEAD (1,000 mg/kg) and imipramine (15 mg/kg) orally respectively. So also, groups 6 and 7 mice were treated with MEAD and imipramine orally respectively. At the end of 1 hour, mice in all groups were assessed using TST (Meizhu *et al*., 2014).

3.3.5.5 To investigate the possible involvement of Cholinergic System

To investigate the possible involvement of the cholinergic system in the antidepressant action of MEAD, 40 mice were divided into 5 groups of 8 mice each were used. The first, second and third groups received distilled water (10 ml/kg), MEAD (1,000mg/kg) and imipramine (15 mg/kg) orally respectively. The fourth and fifth groups were pretreated with atropine (1 mg/kg, *ip*) a muscarinic receptor antagonist 15 minutes prior to administration of MEAD (1,000 mg/kg) and imipramine (15 mg/kg) orally respectively. Mice in all the groups were assessed one hour later using TST.

3.3.5.6 To investigate the possible involvement of Opioidergic System

To investigate the possible involvement of the opioidergic system in the antidepressant action of MEAD, 40 mice were divided into 5 groups of 8 mice each were used. The first, second and third groups received distilled water (10 ml/kg), MEAD (1,000mg/kg) and imipramine (15 mg/kg) orally respectively. The fourth and fifth groups were pretreated with naloxone (2 mg/kg, *ip*) an opioid receptor antagonist 15 minutes prior to administration of MEAD (1,000 mg/kg) and imipramine (15 mg/kg) orally respectively. Mice in all the groups were assessed one hour later using TST.

3.4 Statistical Analysis

Statistical package for social science (SPSS) version 23.0 was used to analyzed data. Results for open field test (OFT), beam walking assay and novel object recognition test (NORT), forced swim test (FST), tail suspension test (TST), BDNF concentration, cortisol level, SOD and MDA concentrations were analyzed using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc test. Data for weights from the chronic unpredictable mild stress was analysed using repeated measures ANOVA followed by Bonferoni post hoc test. Data were presented on tables, figures or plates as percentages and mean ± standard error of mean where necessary. Results were considered significant at $p \leq 0.05$.

CHAPTER FOUR

4.0 RESULTS

4.1 Ethnobotanical Survey

4.1.1 Demography of Respondents in an Ethnobotanical Survey of Medicinal Plants Commonly Used in the Management of Depression in Zaria, Nigeria

A total of ten respondents made up of herb sellers 2 (20%), traditional medical practitioners 6 (60%) and herbalists 2 (20%). Majority (90%) of the respondents were males as observed from demographic information of respondents (Table 4.1).

Table 4.1: Demography of Respondents in an Ethnobotanical Survey of Medicinal Plants Commonly Used in the Management of Depression in Zaria, Nigeria

Parameter	Specification	n(%)
Practice specification	Herb sellers	2(20)
	Traditional medical practitioners	6(60)
	Herbalists	2(20)
Sex	Male	9(90)
	Female	1(10)
Age (years)	20-30	2(20)
	31-40	2(20)
	41-50	4(40)
	51 above	2(20)
Religion	Christianity	0(0)
	Islam	8(80)
	Traditional	2(20)
Nationality	Nigerian	10(100)
	Non Nigerian	0(0)

n= number, %= percentage

4.1.2 Professional Experience of the Respondents during the Ethnobotanical Survey of Medicinal Plants Commonly Used in the Management of Depression in Zaria, Nigeria

The survey showed that only two (20%) of the respondents use incantation and divination to aid the cure of the disease. It was observed that a great percentage (90%) of respondents inherited the knowledge of herbs from their ancestors (Table 4.2).

Table 4.2: Professional Experience of the Respondents During an Ethnobotanical Survey of Medicinal Plants Commonly Used in the Management of Depression in Zaria, Nigeria

Parameter	Specification	n(%)
Frequency of treatment	Regular	8(80)
	Irregular	2(20)
Duration of treatment (days)	2-3	0(0)
	4-15	3(30)
	6-14	7(70)
Other treatment apart from herb	Divination/oracle/incantation	2(20)
	None	8(80)
Source of knowledge	Ancestral	9(90)
	Training	5(5)
	Ancestral/Training	5(5)
	Divination	0(0)
Source of plant/ plant parts	Forest	6(60)
	Home garden	1(10)
	Market	3(30)
Accompanied side effects	Nausea/vomiting	2(20)
	Others	4(40)
	None	4(40)
Accompanied verbal instructions	Yes	10(100)
	No	0(0)

n= number of respondents; % percentage of respondents

4.1.3 Medicinal Plants Commonly Used in the Management of Depression in Zaria Nigeria

The survey also showed that a total of ten medicinal plants species were in use by different categories of practitioners in Zaria. Botanical names, local names, English/common names, family and plant part(s) mentioned are presented in table 4.3.

Table 4.3: Medicinal Plants Commonly Used in the Management of Depression in Zaria, Nigeria

S/No	Botanical name	Local/vernacular name	Voucher number	Family name	Part(s) used
1	*Acacia seyal*	*Dumushi*		Leguminosae	Root
2	*Adansonia digitata*	*Kuka*	2512	Bombaceae	Bark
3	*Caralluma dalzielli*	*Karan masallaci*	217	Asclepiadaceae	Whole
4	*Combretum micranthum*	*Geza*	900257	Combretaceae	Leaves
5	*Ficus platyphylla*	*Gamji*	900106	Moraceae	Bark
6	*Olax manni*	*Tsadar Biri*	1697	Olacaceae	Root
7	*Pancrantum africanum*	*Gadali mai nono*	1203	Amaryllidaceae	Bulbs
8	*Senna occidentalis*	*Rai dore*	1047	Leguminosae	Root
9	*Tapinanthus dodoneifolius* (*Parkia biglobosa*)	*Kaucen Dorawa*	2838 (2846)	Loranthaceae	Whole
10	*Tapinanthus globiferus* (*Vitellaria paradoxa*)	*Kaucen Kade*	1052 (900072)	Loranthaceae	Whole

4.1.4 Plant Forms and Parts Used in the Treatment of Depression by Traditional Medical Practicioner in Zaria, Nigeria

Majority of the plant life forms and parts used are trees and root/bark respectively. Other plant forms and their frequencies were reported (Table 4.4).

Table 4.4: Plant Forms, Parts Used and Frequencies of their Use in the Management of Depression by the Traditional Practicioner in Zaria, Nigeria

Plant life forms	Plant parts used	Frequency of Use
Tree	Root and Bark	6
Shrub	Whole	1
Climber	Whole	2
Creeper	Bulbs	1

4.1.5 Distribution of the Ethnomedicinal Plants Used in the Treatment of Depression by Traditional Medical Practicioner and their Recipes

Most of the recipes were obtained from a single plant source, while others were in combination with other plants. Infusions and grinding were the most frequently used methods. Only three of the recipes were prepared using more than one method (Table 4.5).

Table 4.5: Distribution of the Ethnomedicinal Plants Used in the Treatment of Depression and Recipes

S/No	Taxa	Traditional solvent of choice	Method of preparation	Method of administration
1	*Acacia seyal*	Water	Infusion	Decoction taken 2-3 times daily
2	*Adansonia digitata*	Water	Infusion/ powder	3 finger full powder taken with milk
3	*Caralluma dalzielli*	Water	Grinding	Whole plants squeezed are taken in low quantity once daily
4	*Combretum micranthum*	Water/ Alcohol	Powder	Decoction 2-3 times daily
5	*Ficus platyphylla*	Water	Decoction/ maceration	Take decoction in low quantity twice daily
6	*Olax manni*	Water/Pure honey	Infusion	Roots boiled, half-cup taken orally
7	*Pancrantium africanum*	Water	Infusion	Half a cup, morning and night
8	*Senna occidentalis*	Water/ Honey	Decoction	Extracts taken twice daily
9	*Tapinanthus dodeinofolius*	Water/ Fresh milk	Grinding/ powder	Powder taken with milk
10	*Tapinanthus globiferus*	Water	Grinding/ powder	Powder taken with pap, Fluid are taken orally

4.2 Phytochemical Constituents

4.2.1 Phytochemical Constituents present in the Methanol Extracts of the Ethnomedicinal Plants Used in the Management of Depression

The phytochemical constituents present in the methanol extracts of the plants used by the traditional practicioner in Zaria for the management of depression include glycosides, carbohydrates and alkaloids. Phytochemical constituents like anthraquinones, flavonoids and tannins were also present in some of the plants (Table 4.6). The thin layer chromatogram corroborating the above are shown in plates I-VI.

Table 4.6: Phytochemical Constituents present in the Methanol Extracts of the Medicinal Plants Used in the Management of Depression in Zaria Nigeria

S/No	Phytoconstituents	Methanol Extracts								
		TdD	**TdV**	**Ad**	**Fp**	**Om**	**So**	**Cd**	**Pa**	**Cm**
1	Alkaloids	+	+	+	+	+	+	+	+	+
2	Flavonoids	+	-	+	+	+	+	+	+	+
3	Tannins	+	-	+	+	+	-	+	+	+
4	Saponin glycoside	+	+	+	+	+	-	+	+	+
5	Cardiac glycoside	+	+	+	+	+	+	+	+	+
6	Unsaturated Steroids	-	+	+	+	+	-	+	+	+
	& Triterpenes	+	+	+	-	+	-	+	-	+
7	Anthraquinones	-	-	+	+	-	+	-	-	-
8	Carbohydrates	+	+	+	+	+	+	+	+	+

TdD=*Tapinanthus dodoneifolius* (Host name= *Parkia biglobosa*), **TdV**= *Tapinanthus globiferus* (Host name= *Vitellaria paradoxa*), **Fp**= *Ficus platyphylla*, **Ad**= *Adansonia digitata*, **Om**= *Olax manni*, **So**= *Senna occidentalis*, **Cd**= *Caralluma dalzielli*, **Pa**= *Pancrantum africanum*, **Cm**= *Combretum micranthum*

+ = present, - = absent

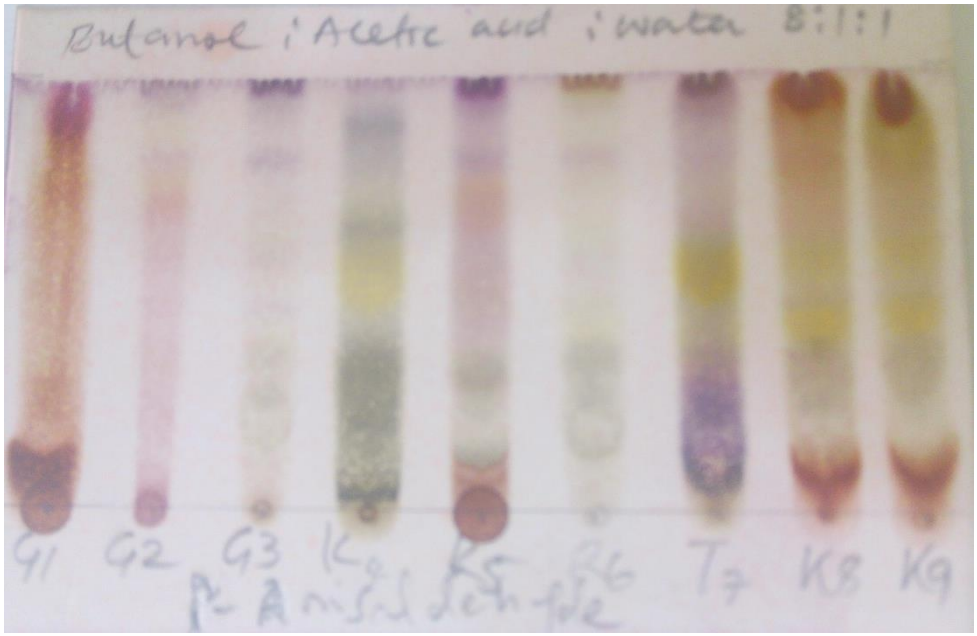


Plate I: Thin Layer Chromatogram of the Various Methanol Extracts of the Surveyed Plants Developed in Butanol: Acetic acid: Water (8:1:1) and Sprayed with P-anisaldehyde Reagent

G1=*Ficus platyphylla*; G2=*Combretum micranthum*; G3=*Pancrantum africanum*; K4=*Caralluma dalzielli*; K5= *Adansonia digitata*; R6=*Senna occidentalis*; T7= *Olax manni*; K8= *Tapinanthus dodeinofolius* (*Parkia biglobosa*); and K9= *Tapinanthus globiferus* (*Vitellaria paradoxa*)

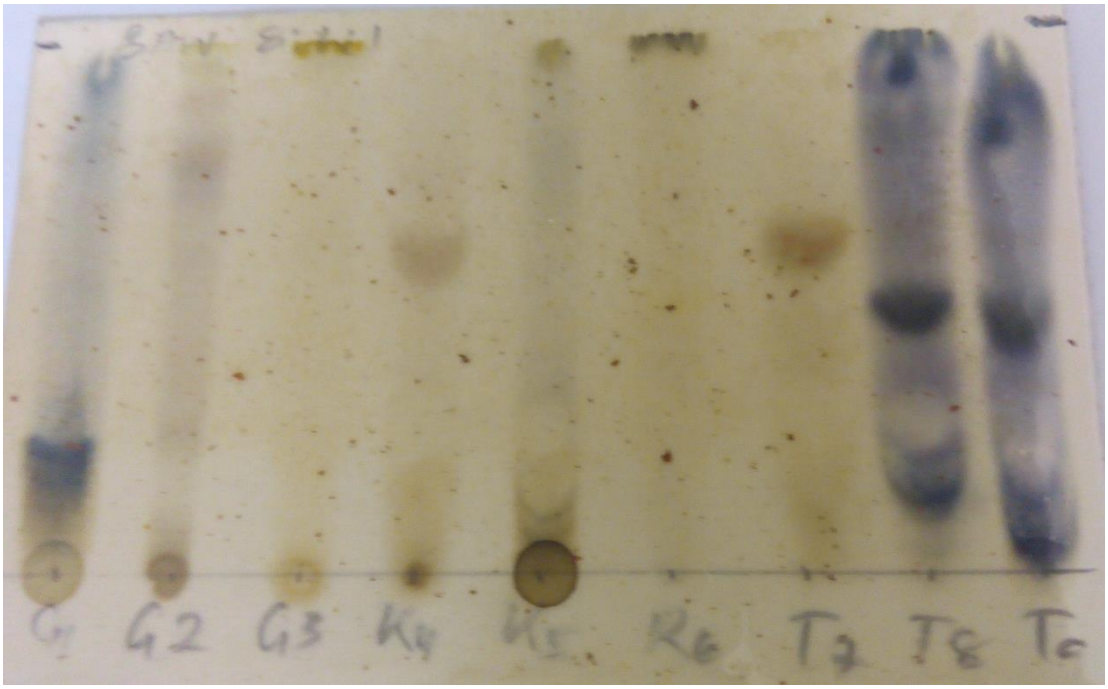


Plate II: Thin Layer Chromatogram of the Various Methanol Extracts of the Surveyed Plants Developed in Butanol: Acetic acid: Water (8:1:1) and Sprayed with Ferric Chloride Solution

G1= *Ficus platyphylla*; G2= *Combretum micranthum*; G3= *Pancrantum africanum*; K4= *Caralluma dalzielli*; K5= *Adansonia digitata*; R6= *Senna occidentalis*; T7= *Olax manni*; T8= *Tapinanthus globiferus* (*Parkia biglobosa*); and T9= *Tapinanthus dodoneifolius* (*Vitellaria paradoxa*)

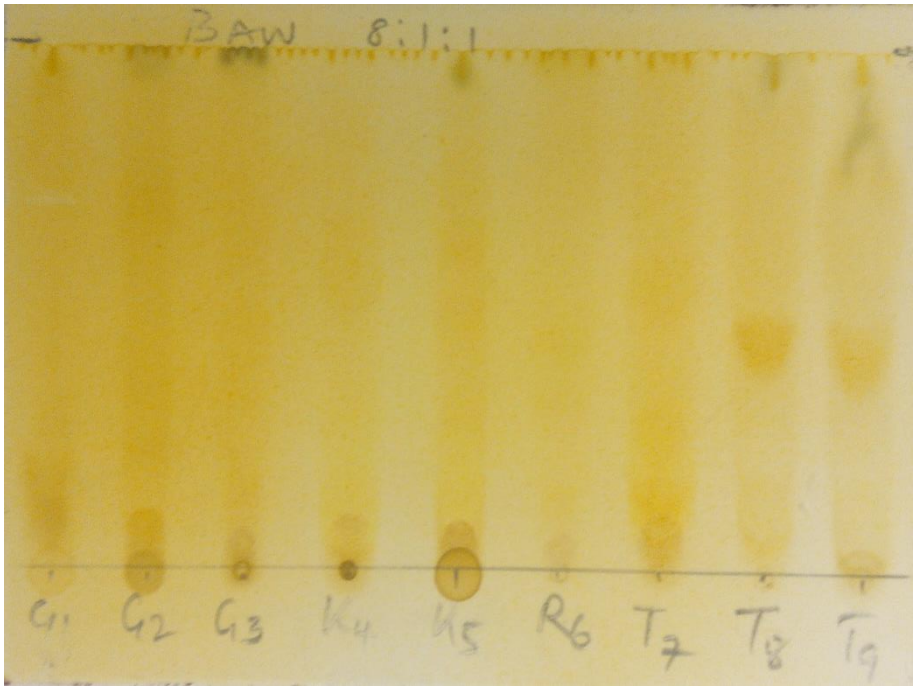


Plate III: Thin Layer Chromatogram of the Various Methanol Extracts of the Surveyed Plants developed in Butanol: Acetic acid: Water (8:1:1) and sprayed with Dragendorff's Reagent

G1= *Ficus platyphylla*; G2= *Combretum micranthum*; G3=*Pancrantum africanum*; K4= *Caralluma dalzielli*; K5= *Adansonia digitata*; R6= *Senna occidentalis*; T7= *Olax manni*; T8= *Tapinanthus globiferus* (*Parkia biglobosa*); and T9= *Tapinanthus dodoneifolius* (*Vitellaria paradoxa*)

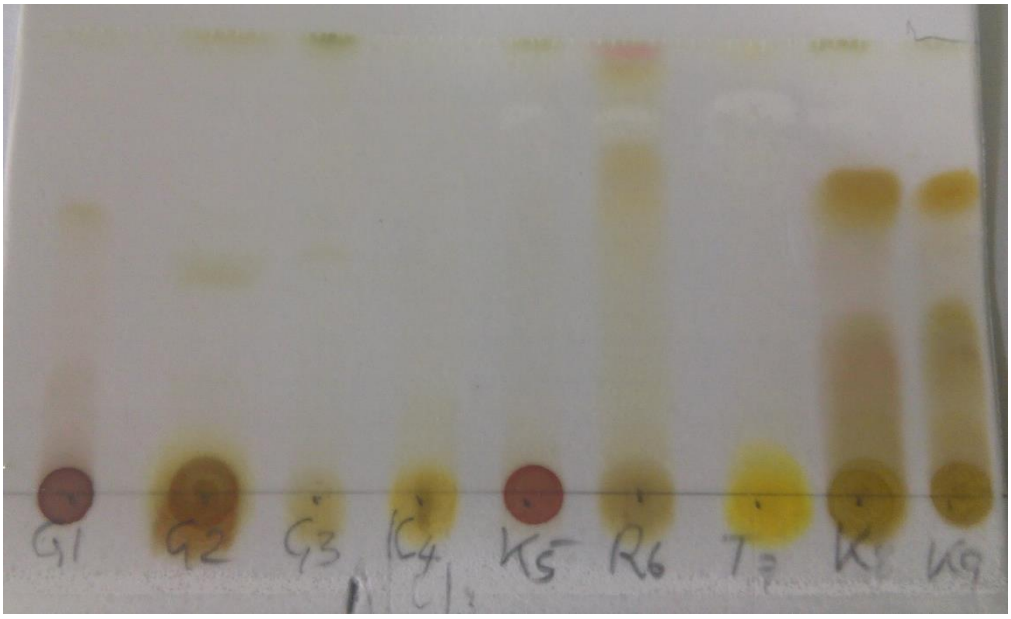


Plate IV: Thin Layer Chromatogram of the Various Methanol Extracts of the Surveyed Plants Developed in Butanol: Acetic acid: Water (8:1:1) and Sprayed with Aluminium chloride Solutio

G1= *Ficus platyphylla*; G2= *Combretum micranthum*; G3=*Pancrantum africanum*; K4= *Caralluma dalzielli*; K5= *Adansonia digitata*; R6= *Senna occidentalis*; T7= *Olax manni*; T8= *Tapinanthus globiferus* (*Parkia biglobosa*); and T9= *Tapinanthus dodoneifolius* (*Vitellaria paradoxa*)

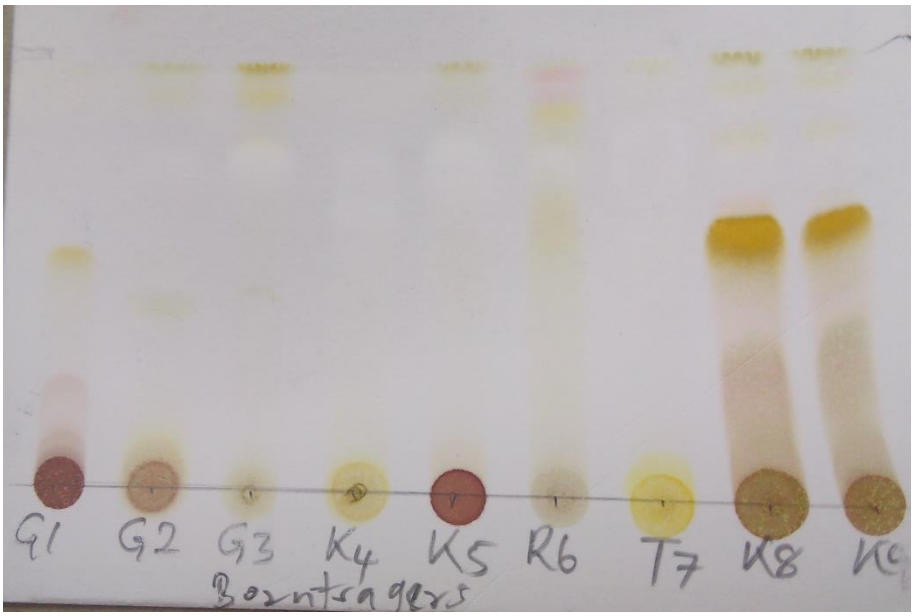


Plate V: Thin Layer Chromatogram of the Various Methanol Extracts of the Surveyed Plants Developed in Butanol: Acetic acid: Water (8:1:1) and Sprayed with Borntrager's Reagent

G1= *Ficus platyphylla*; G2= *Combretum micranthum*; G3=*Pancrantum africanum*; K4= *Caralluma dalzielli*; K5= *Adansonia digitata*; R6= *Senna occidentalis*; T7= *Olax manni*; K8= *Tapinanthus globiferus* (*Parkia biglobosa*); and K9= *Tapinanthus dodoneifolius* (*Vitellaria paradoxa*)

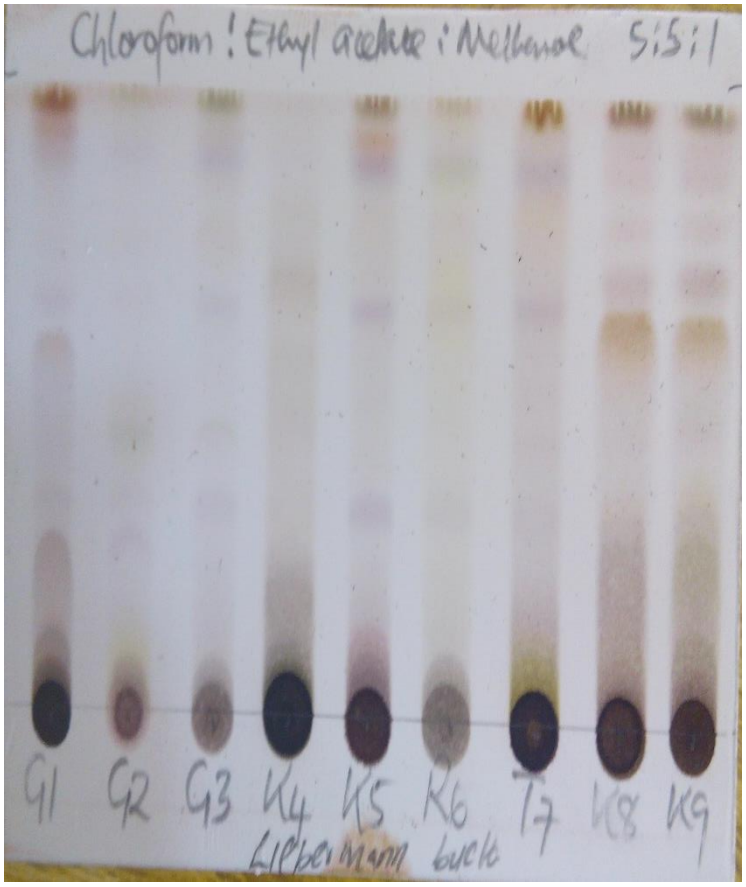


Plate VI: Thin Layer Chromatogram of the Various Methanol Extracts of the Surveyed Plants Developed in Chloroform: Ethylacetate: Methanol (5:5:1) and Sprayed with Liebermann-Burchard's Reagent

G1= *Ficus platyphylla*; G2= *Combretum micranthum*; G3=*Pancrantum africanum*; K4= *Caralluma dalzielli*; K5= *Adansonia digitata*; R6= *Senna occidentalis*; T7= *Olax manni*; K8= *Tapinanthus globiferus* (*Parkia biglobosa*); and K9= *Tapinanthus dodoneifolius* (*Vitellaria paradoxa*)

4.3 Acute and Neurotoxicity Studies

4.3.1 Median Lethal Dose (LD_{50}) Values of the Methanol Extracts of the Medicinal Plants Used in the Management of Depression in Zaria

The methanol extract of the medicinal plants *Adansonia digitata*, *Acacia seyal*, *Tapinanthus dodeinofolius*, *Tapinanthus globiferus*, *Pancratium africanum*, *Senna occidentalis* and *Pancrantum africanum* had LD_{50} estimated to be greater than 5,000 mg/kg orally in mice from the limit test (Table 4.6a). However, plants like *Ficus platyphylla*, *Caralluma dalzielli* and *Olax manni* had LD_{50} ≥2,000 mg/kg from the main test, with signs and symptoms of toxicity reported from the limit test (Table 4.6b).

Table 4.6a: Median Lethal Dose (LD_{50}) Values for Methanol Extracts of Medicinal Plants Used in the Management of Depression in Zaria

S/No	Methanol Extracts	LD_{50} Values (mg/kg)
1	*Tapinanthus dodoneifolius* (Host= *Parkia biglobosa*)	≥5,000
2	*Tapinanthus globiferus* (Host= *Vitellaria paradoxa*)	≥5,000
3	*Pancrantum africanum*	≥5,000
4	*Adansonia digitata*	≥5,000
5	*Combretum micranthum*	≥5,000
6	*Senna occidentalis*	≥5,000

n=3.

Table 4.6b: Median Lethal Dose (LD_{50}) Values of Methanol Extract of Medicinal Plants Used in the Management of Depression in Zaria

Plants	LD_{50} Values	Onset of toxicity	Duration of toxicity	Signs and symptoms of toxicity
Fp, Cd, Om (Limit test)	≤5,000	Immediately	<24 hours	Coma, Death
Ficus platyphylla	≥2,000	Nil	Nil	Nil
Caralluma dalzielli	≥2,000	Nil	Nil	Nil
Olax manni	≥2,000	Nil	Nil	Nil

n=3 for limit test; n=5 for main test, Fp= *Ficus platyphylla*; Cd= *Caralluma dalzielli*; Om= *Olax manni*

4.3.2 Neurotoxicity Studies of the Methanol Extracts of the Medicinal Plants Used by Traditional Practicioners in Management of Depression

When mice treated with methanol extracts of all the medicinal plants used in the management of depression by traditional medical practicioners in Zaria when subjected to beam walking assay, there was no significant increase in number of foot slips at all the three doses tested orally. The standard drug, diazepam, significantly ($p \leq 0.001$) increased the number of foot slips at the dose of 10 mg/kg orally (Table 4.7).

Table 4.7: Effect of Methanol Extract of the Medicinal Plants Used by Traditional Practicioners in Management of Depression on Motor Coordination in Mice

Treatment	Dose (mg/kg, *po*)	Mean number of foot slips
Distilled water	10 ml/kg	2.00 ± 0.0.37
Diazepam	10	7.17 ± 1.58*
Adansonia digitata	250	0.67 ± 0.42
	500	0.33 ± 0.21
	1000	1.67 ± 0.21
Combretum micranthum	250	0.00 ± 0.0
	500	1.00 ± 0.63
	1000	0.33 ± 0.21
Caralluma dalzielli	125	0.00 ± 0.00
	250	0.00 ± 0.00
	500	0.67 ± 0.21
Ficus platyphylla	125	0.00 ± 0.00
	250	1.00 ± 0.37
	500	0.33 ± 0.21
Olax manni	125	0.67 ± 0.21
	250	0.00 ± 0.00
	500	0.67 ± 0.21
Pancrantum micranthum	250	0.33 ± 0.21
	500	0.67 ± 0.21
	1000	0.83 ± 0.17
Senna occidentalis	250	0.67 ± 0.21
	500	0.33 ± 0.21
	1000	0.67 ± 0.21
Tapinanthus dodeinofolius	250	1.67 ± 0.76
	500	0.33 ± 0.21
	1000	1.67 ± 0.76
Tapinanthus globiferus	250	0.33 ± 0.21
	500	0.00 ± 0.00
	1000	0.67 ± 0.21

Data presented as mean±SEM. Data was analyesd using Oneway ANOVA followed by Bonferoni post hoc test. *$p \leq 0.01$ significant difference as compared to distilled water group.

4.4 Preliminary Antidepressant Studies

4.4.1 Effects of Methanol Extracts of the Medicinal Plants Used by Traditional Practitioners in the Management of Depression on Tail Suspension Test in Mice

The results of this test revealed that the duration immobility of the mice treated with the various plant extract decreased in a dose-dependent manner except for *Tapinanthus dodeinofolius, Tapinanthus globiferus, Olax manni, Combretum micranthum* and *Senna occidentalis*, they showed dose independent decrease of duration of immobility. Significant decrease in duration of immobility was obtained at all the tested doses ($p \leq 0.05$) as compared to the distilled water (10 ml/kg) group. The standard drug in this test, imipramine (15 mg/kg), also significantly decreased ($p \leq 0.05$) the duration immobility time (Figures 4.1- 4.9).

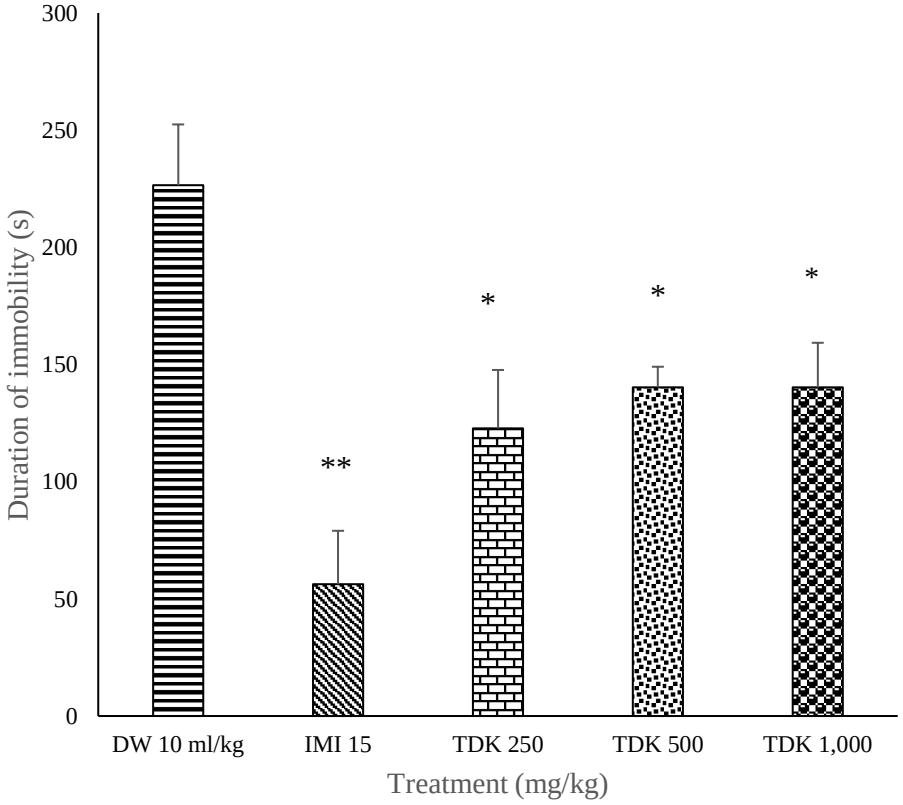


Figure 4.1: Effect of Methanol Extract of *Tapinanthus globiferus* on Duration Immobility of Mice in Tail Suspension Test

Animals were acutely treated with TDK (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferoni post hoc test, *$p \leq 0.01$, **$p \leq 0.001$ significantly different from distilled water treated group. TDK= *Tapinanthus globiferus*, DW= Distilled water, IMI= Imipramine

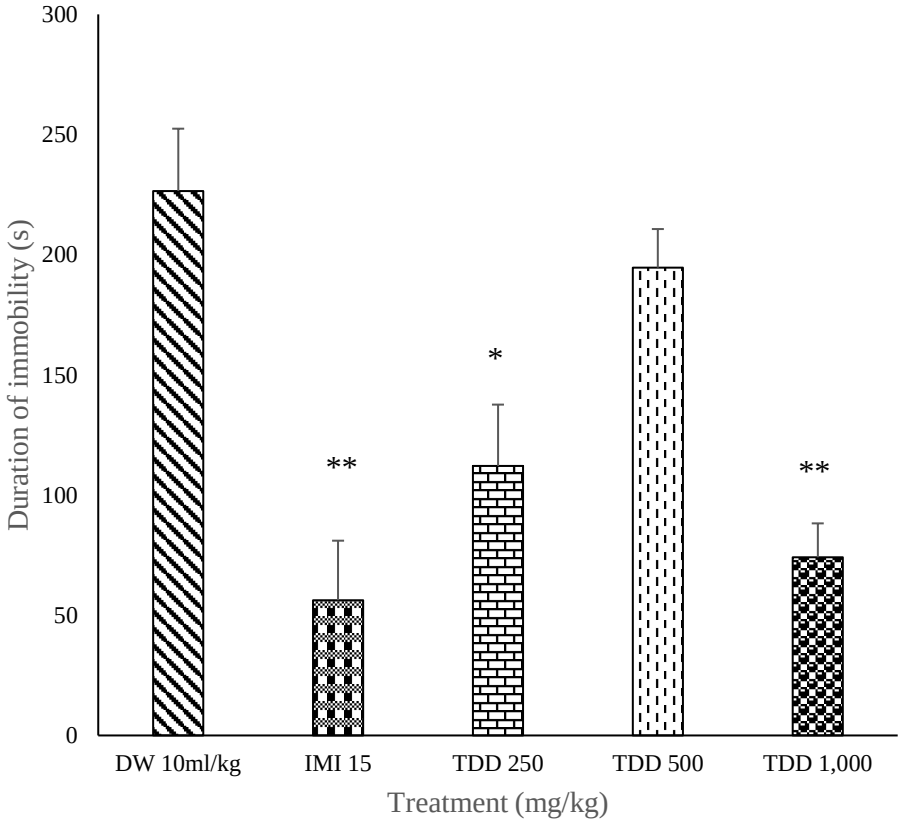


Figure 4.2: **Effect of Methanol Extract of *Tapinanthus dodeinofolius* on Duration Immobility of Mice in Tail Suspension Test**

Animals were acutely treated with TDD (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferoni post hoc test, *$p \leq 0.01$, **$p \leq 0.001$ significantly different from distilled water treated group. TDD= *Tapinanthus dodeinofolius*, DW= Distilled water, IMI= Imipramine

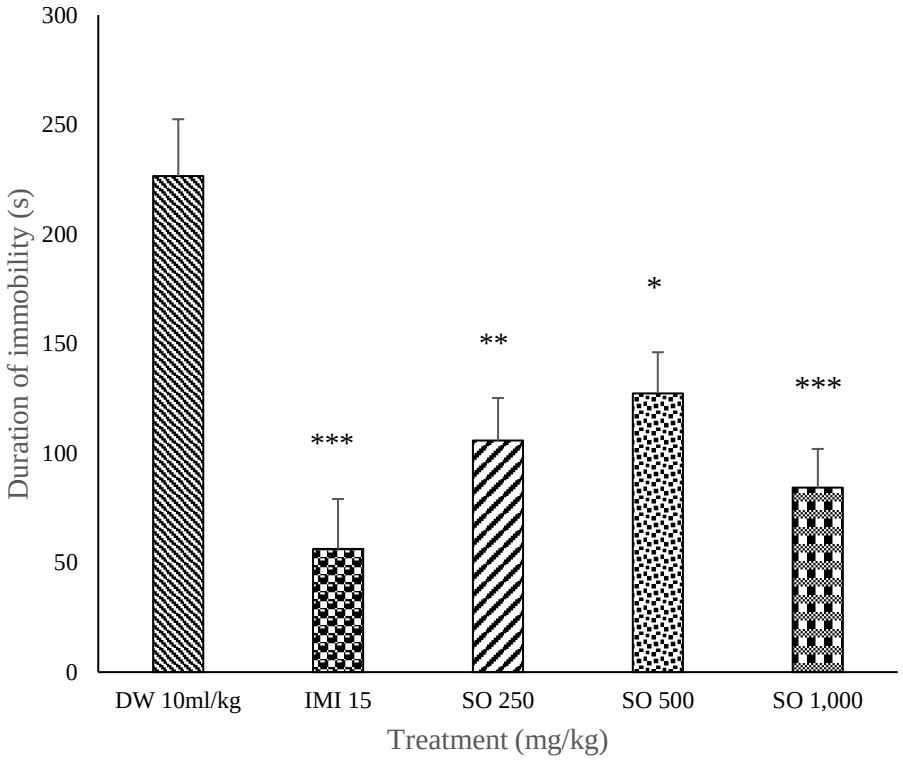


Figure 4.3: **Effect of Methanol Root Extract of *Senna occidentalis* on Duration Immobility of Mice in Tail Suspension Test**

Animals were acutely treated with SO (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± S.E.M. of 8 animals. Data was analysed using one-way ANOVA followed by Bonferoni post hoc test, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ significantly different from distilled water treated group. SO= *Senna occidentalis*, DW= Distilled water, IMI= Imipramine

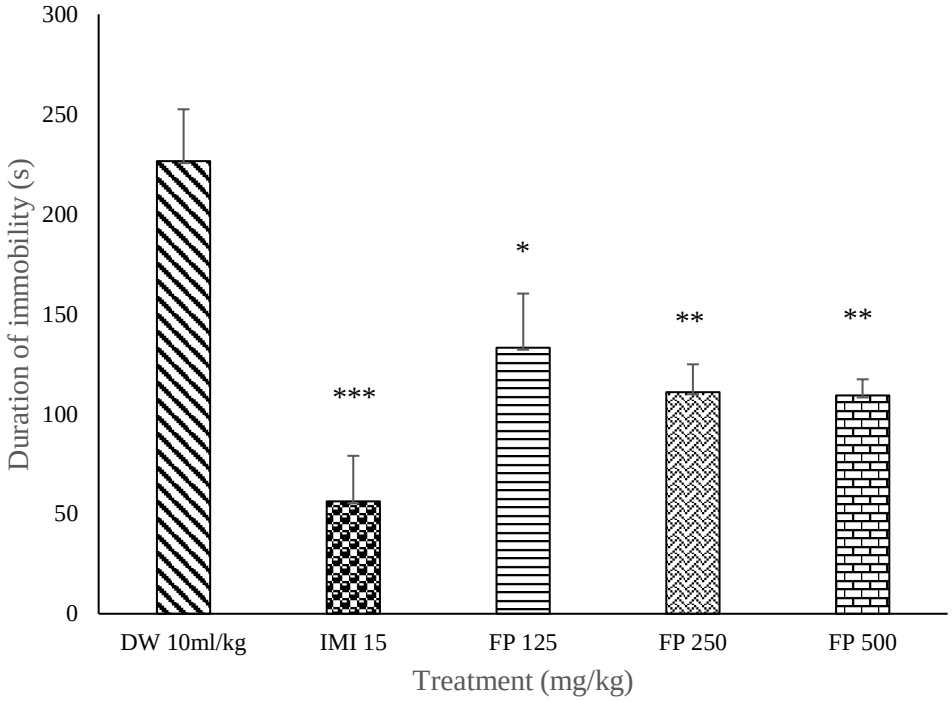


Figure 4.4: **Effect of Methanol Stem Bark Extract of *Ficus platyphylla* on Duration Immobility of Mice in Tail Suspension Test**

Animals were acutely treated with FP (125, 250 or 500 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferoni post hoc test, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ significantly different from distilled water treated group. FP= *Ficus platyphylla*, DW= Distilled water, IMI= Imipramine

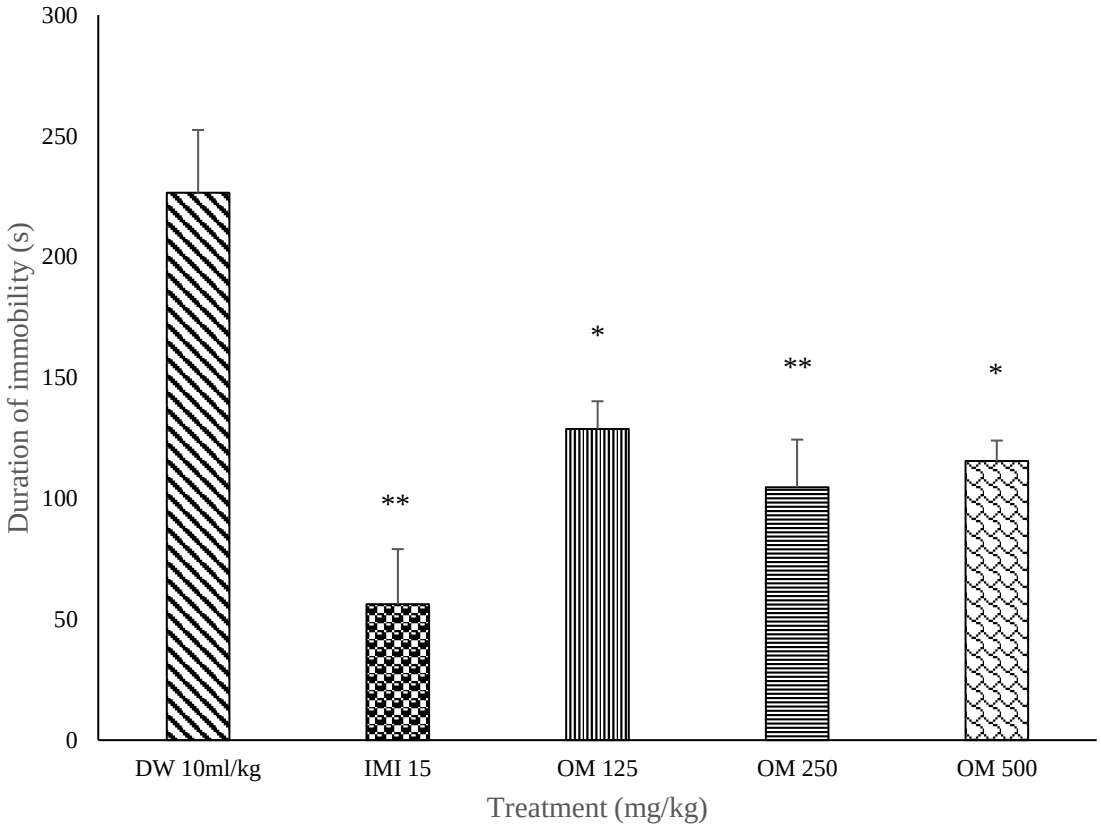


Figure 4.5: **Effect of Methanol Root Extract of *Olax manni* on Duration Immobility of Mice in Tail Suspension Test**

Animals were acutely treated with OM (125, 250 or 500 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferoni post hoc test, *$p \leq 0.01$, **$p \leq 0.001$ significantly different from distilled water treated group. OM= *Olax manni*, DW= Distilled water, IMI= Imipramine

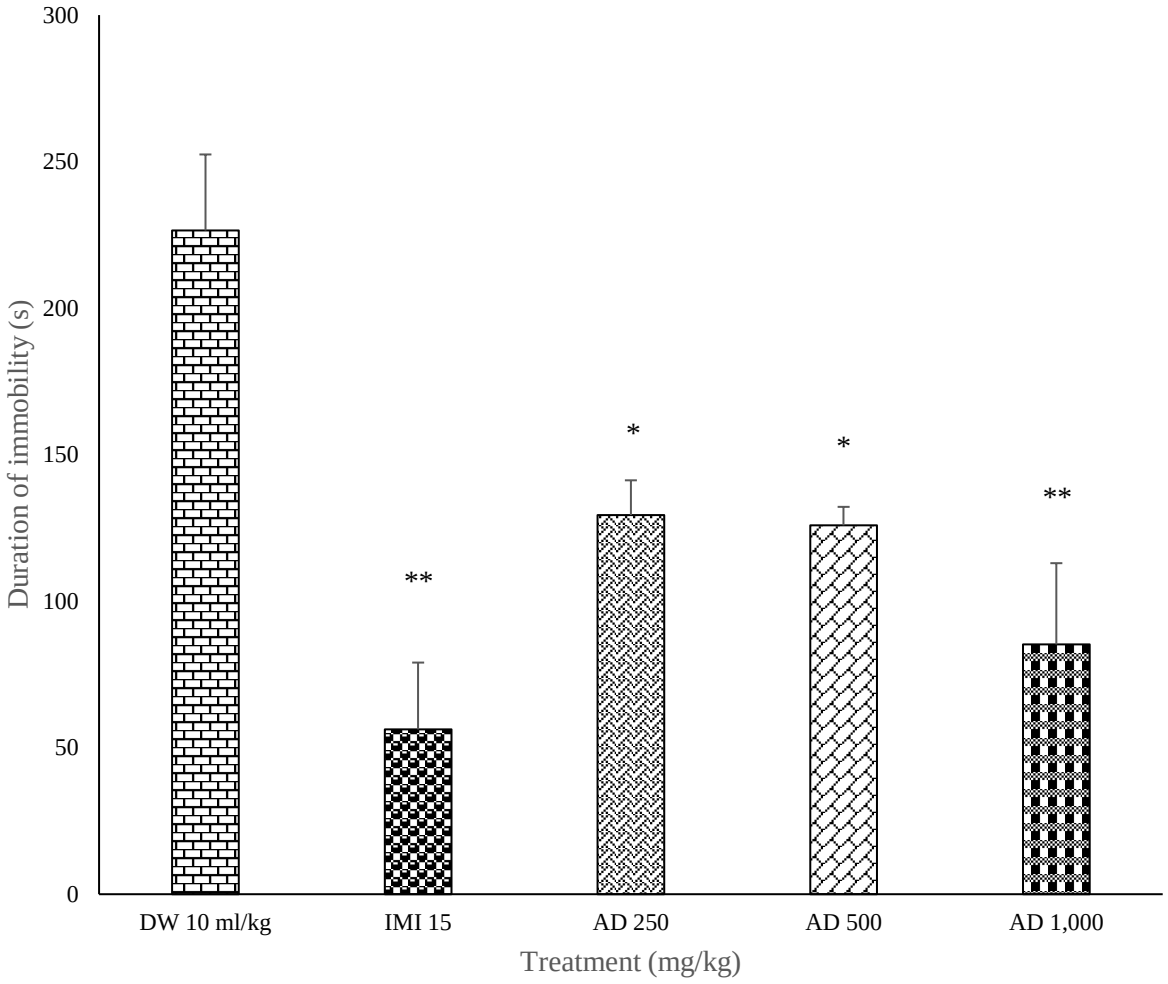


Figure 4.6: **Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Duration Immobility of Mice in Tail Suspension Test**

Animals were acutely treated with AD (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.01$, **$p \leq 0.001$ significantly different from distilled water treated group. AD= *Adansonia digitata*, DW= Distilled water, IMI= Imipramine

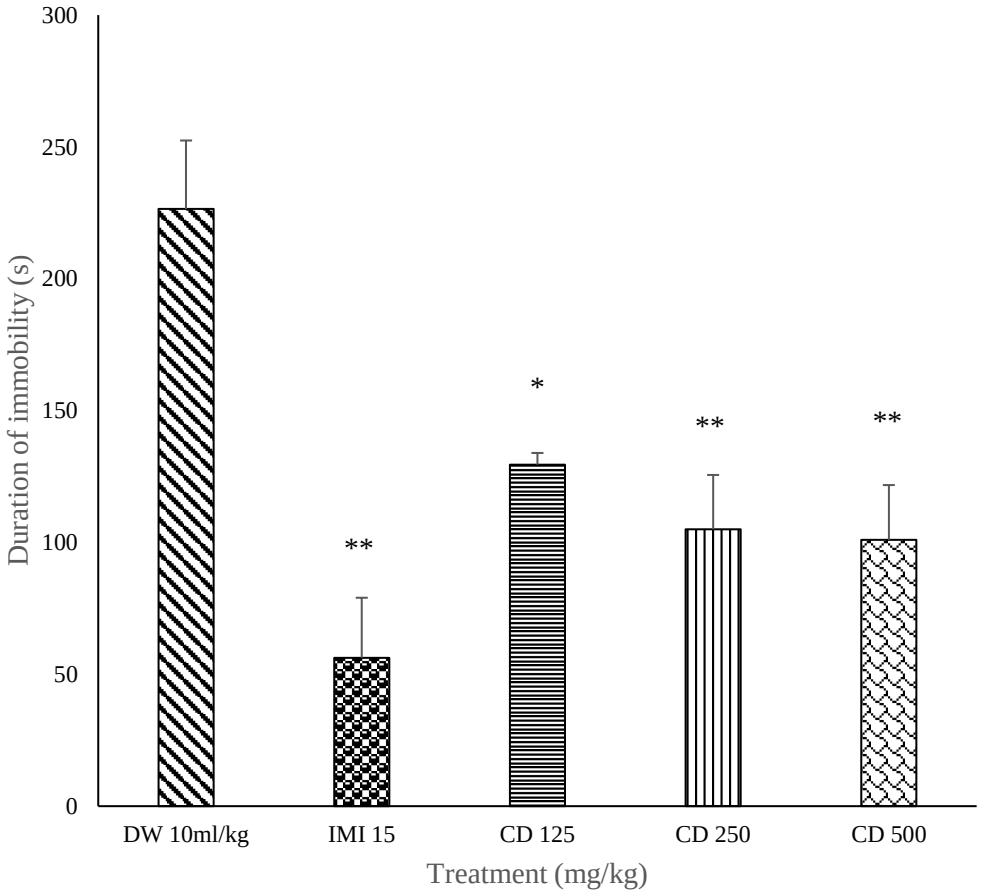


Figure 4.7: **Effect of Methanol Extract of *Caralluma dalzielli* on Duration Immobility of Mice in Tail Suspension Test**

Animals were acutely treated with CD (125, 250 or 500 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.05$, **$p \leq 0.001$ significantly different from distilled water treated group. CD= *Caralluma dalzielli*, DW= Distilled water, IMI= Imipramine

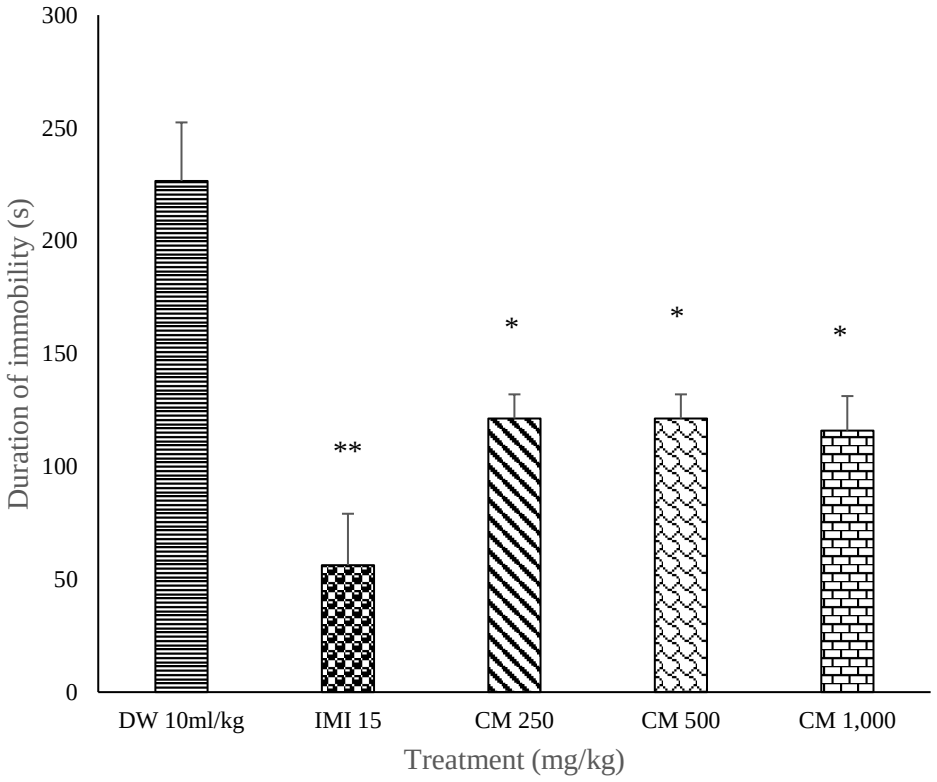


Figure 4.8: **Effect of Methanol Leaf Extract of *Combretum micranthum* on Duration Immobility of Mice in Tail Suspension Test**

Animals were acutely treated with CM (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferoni post hoc test, *$p \leq 0.01$, **$p \leq 0.001$ significantly different from distilled water treated group. CM= *Combretum micranthum*, DW= Distilled water, IMI= Imipramine

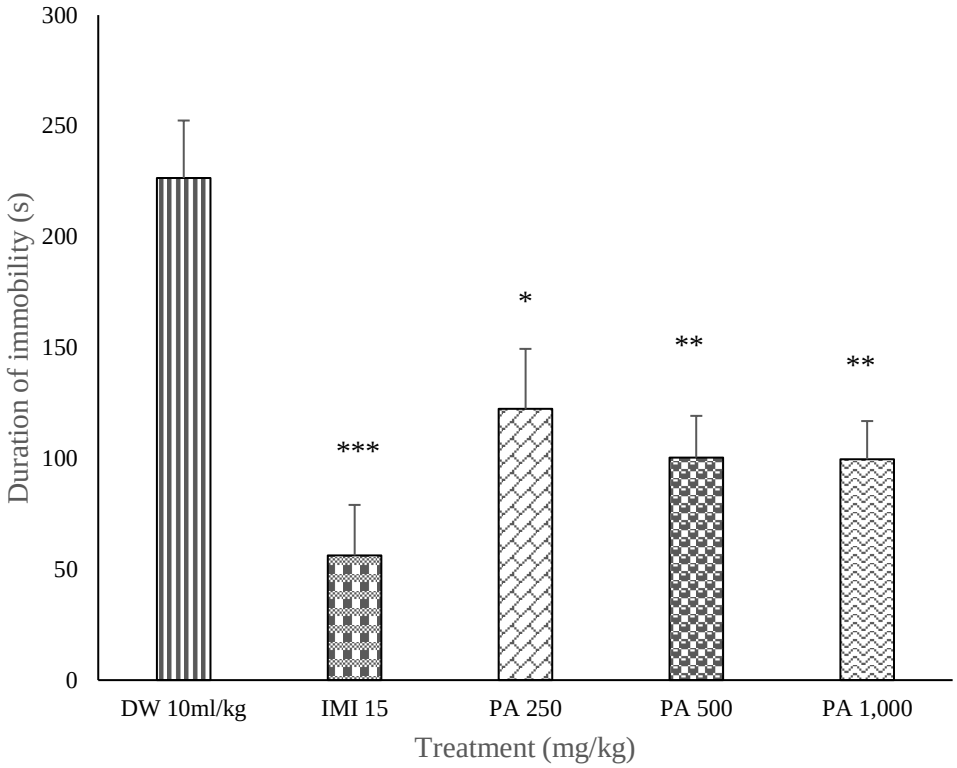


Figure 4.9: **Effect of Methanol Bulbs Extract of *Pancrantium africanum* on Duration Immobility of Mice in Tail Suspension Test**

Animals were acutely treated with PA (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ significantly different from distilled water treated group. PA= *Pancrantium africanum*, DW= Distilled water, IMI= Imipramine

4.4.2 Effects of Methanol Extracts of the Medicinal Plants Used by Traditional Practitioners in the Management of Depression on Open Field Test in Mice

When placed in the open field arena, animals in the control group exhibited ambulatory activity represented by the number of line crossing (108.75 ± 8.39). Pretreatment with the methanol extracts of the medicinal plants collected showed no significant change in ambulatory activity of the mice. However, a significant ($p \leq 0.01$) reduction in locomotor activity was observed with diazepam (10 mg/kg). Additionally, there was significant ($p \leq 0.05$) redction in locomotor activity at certain doses shown by some plants like *Tapinanthus dodeinofolius* (250 mg/kg), *Tapinanthus globiferus* (1,000 mg/kg), *Senna occidentalis* (500 & 1,000 mg/kg) and 250 mg/kg of *Caralluma dalzielli* (Figures 4.10-4.18).

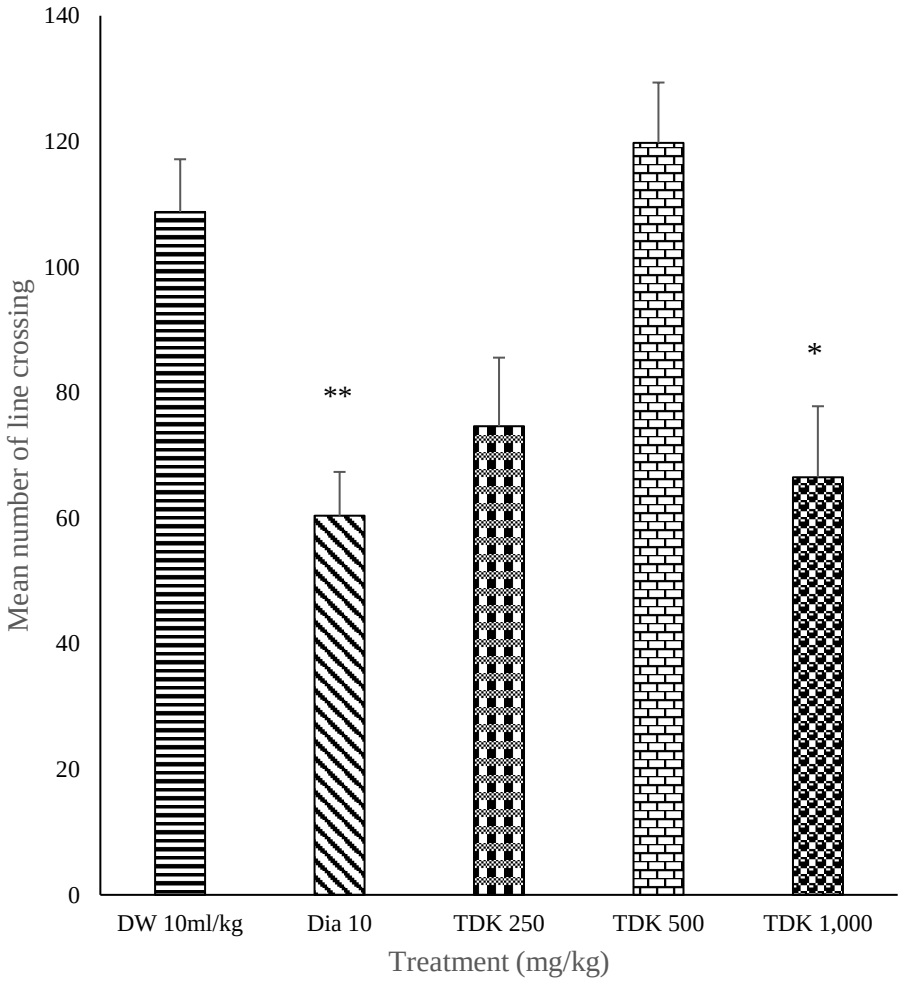


Figure 4.10: **Effect of Methanol Extract of *Tapinanthus globiferus* on Line Crossing of Mice in the Open Field Test**

Animals were acutely treated with the TDK (250, 500, or 1,000mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (10 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.05$; **$p \leq 0.01$ significantly different from distilled water treated animals. TDK= *Tapinanthus globiferus*, DW= Distilled water, Dia= Diazepam

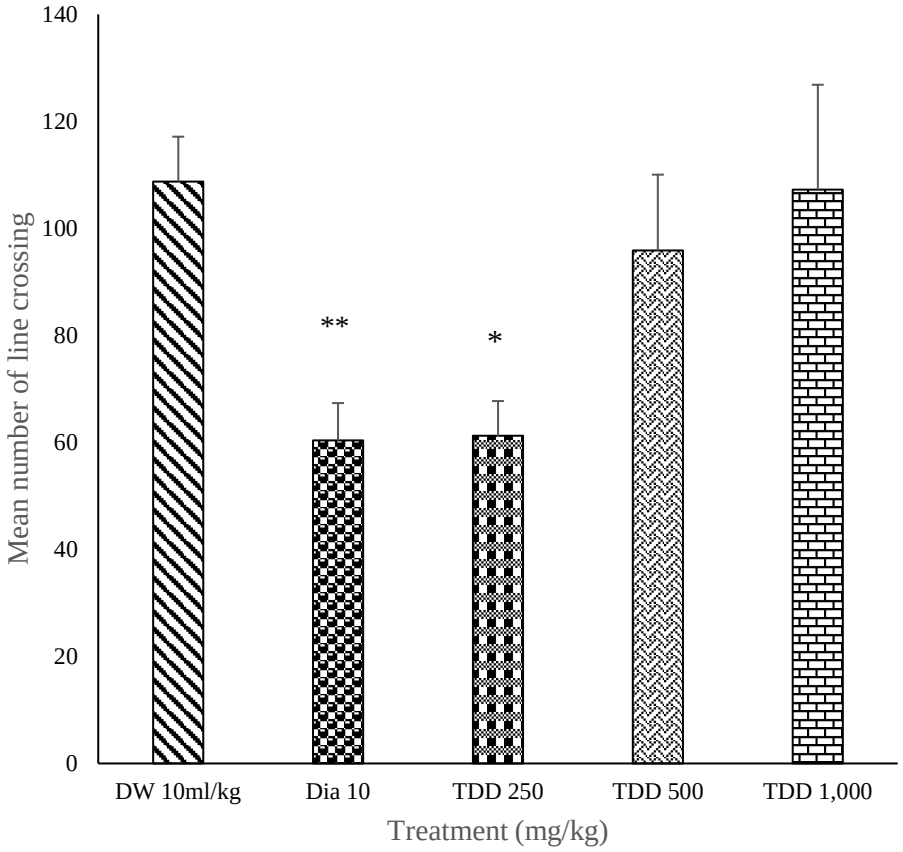


Figure 4.11: **Effect of Methanol Stem Extract of *Tapinanthus dodeinofolius* on Line Crossing of Mice in the Open Field Test**

Animals were acutely treated with TDD (250, 500, or 1,000mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (10 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.05$; **$p \leq 0.01$ significantly different from distilled water treated animals. TDD= *Tapinanthus dodeinofolius*, DW= Distilled water, Imip= Imipramine

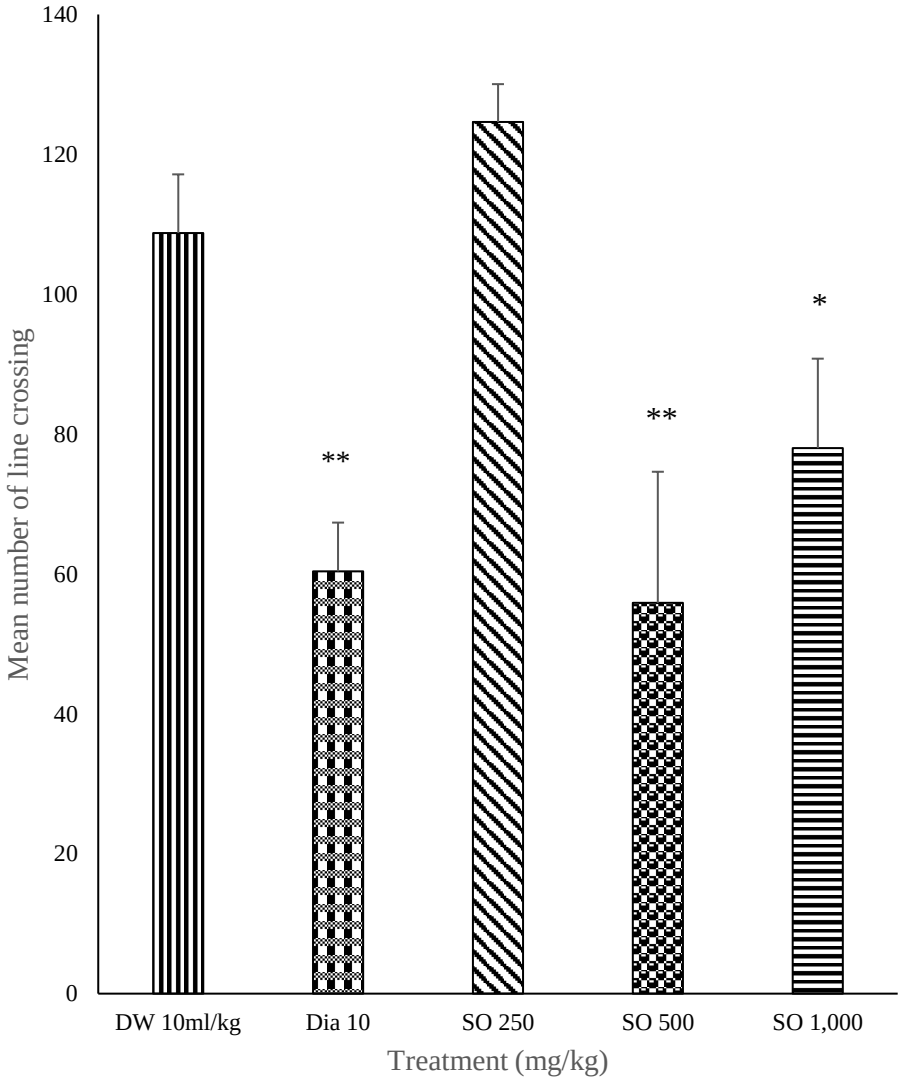


Figure 4.12: **Effect of Methanol Root Extract of *Senna occidentalis* on Line Crossing of Mice in the Open Field Test**

Animals were acutely treated with SO (250, 500, or 1,000mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (10 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.05$; **$p \leq 0.01$ significantly different from distilled water treated animals. SO= *Senna occidentalis*, DW= Distilled water, Dia= Diazepam

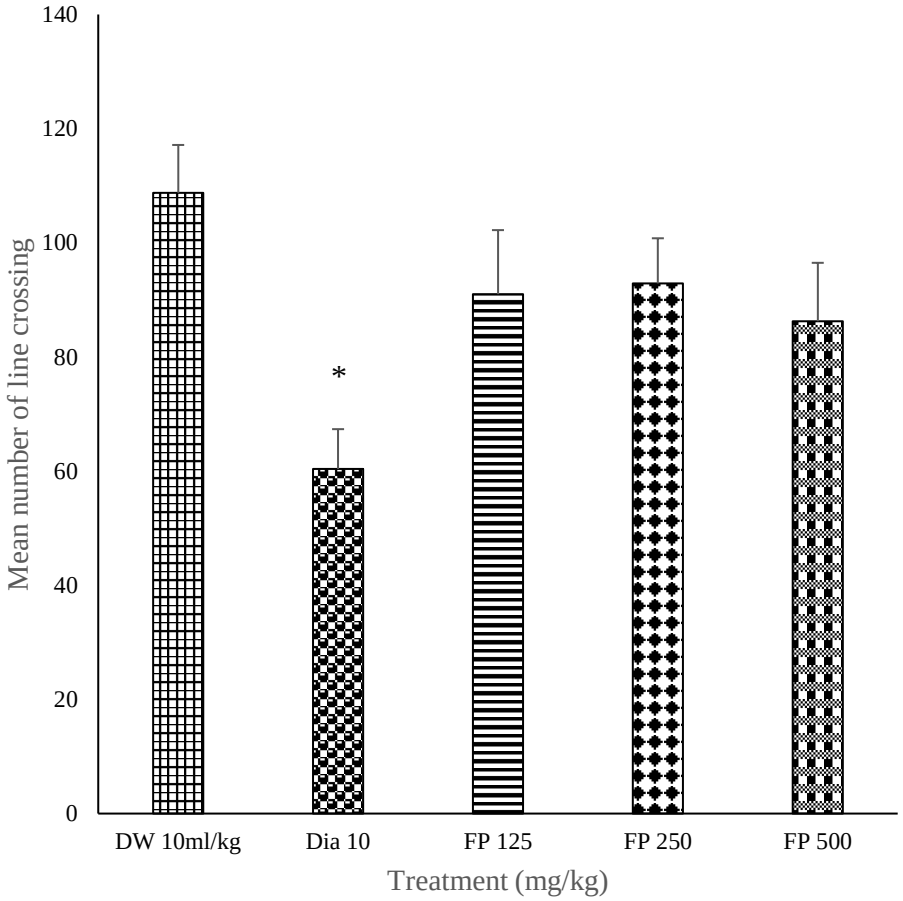


Figure 4.13: **Effect of Methanol Stem Bark Extract of *Ficus platyphylla* on Line Crossing of Mice in the Open Field Test**

Animals were acutely treated with FP (125, 250, or 500 mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (10 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.01$ significantly different from distilled water treated animals. FP= *Ficus platyphylla*, DW= Distilled water, Dia= Diazepam

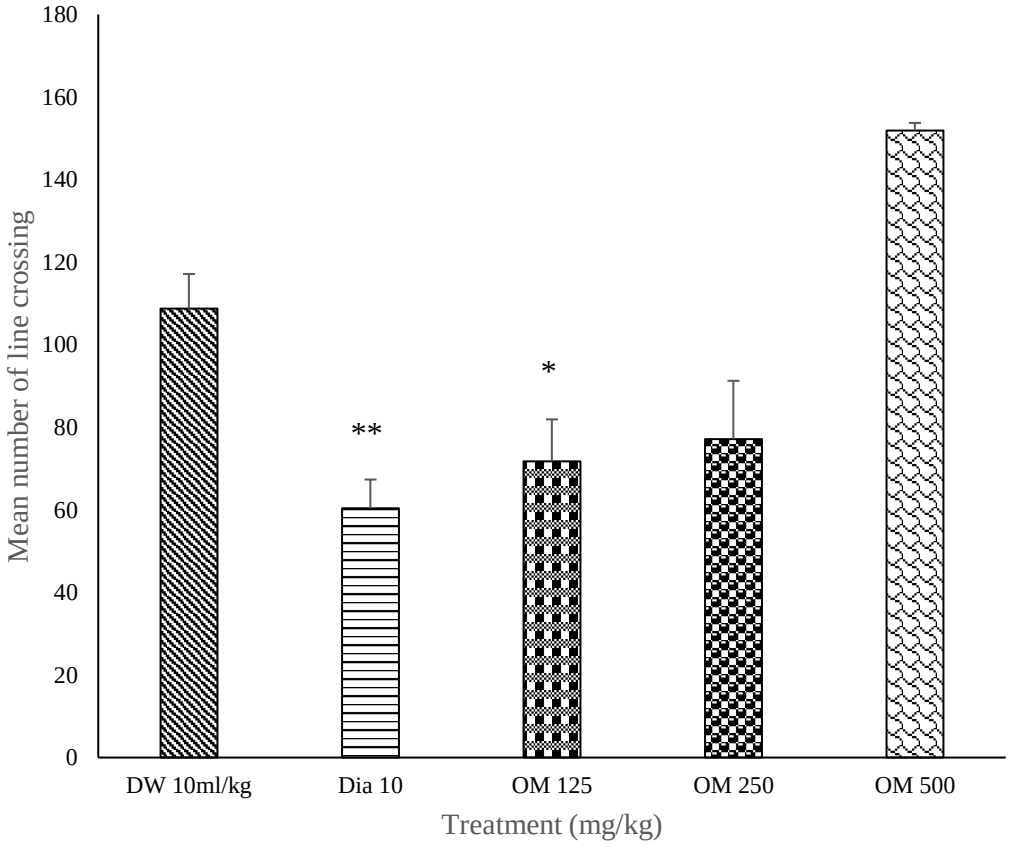


Figure 4.14: **Effect of Methanol Root Extract of *Olax manni* on Line Crossing of Mice in the Open Field Test**

Animals were acutely treated with OM (125, 250, or 500 mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (10 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.05$; **$p \leq 0.01$ significantly different from distilled water treated animals. OM= *Olax manni*, DW= Distilled water, Dia= Diazepam

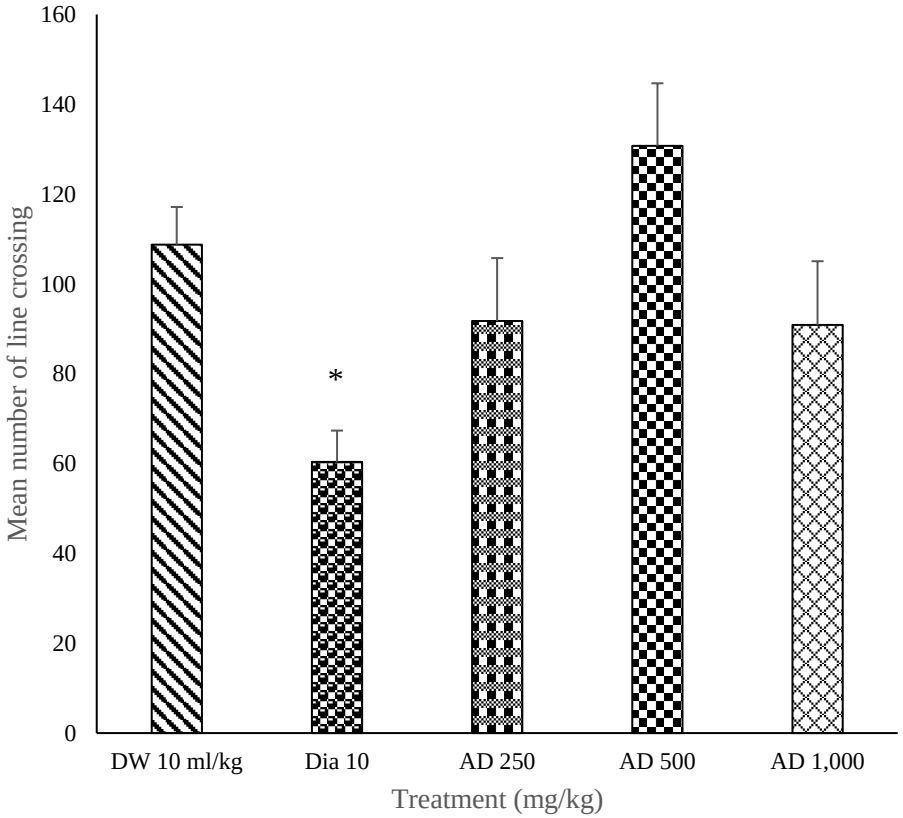


Figure 4.15: **Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Line Crossing of Mice in the Open Field Test**

Animals were acutely treated with AD (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (10 mg/kg, *po*). Each column represents the mean ± S.E.M. of 8 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.01$ significantly different from distilled water treated animals. AD= *Adansonia digitata*, DW= Distilled water, Dia= Diazepam

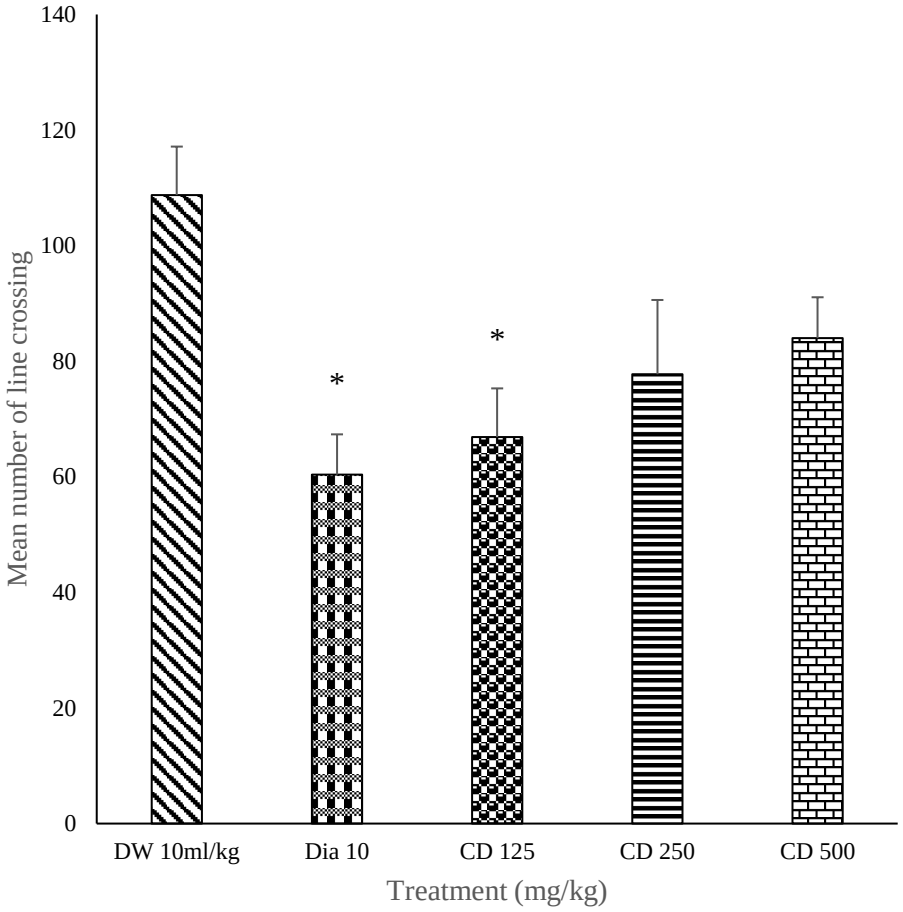


Figure 4.16: **Effect of Methanol Extract of *Caralluma dalzielli* on Line Crossing of Mice in the Open Field Test**

Animals were acutely treated with CD (125, 250, or 500 mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (10 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.01$, significantly different from distilled water treated animals. CD= *Caralluma dalzielli*, DW= Distilled water, Dia= Diazepam

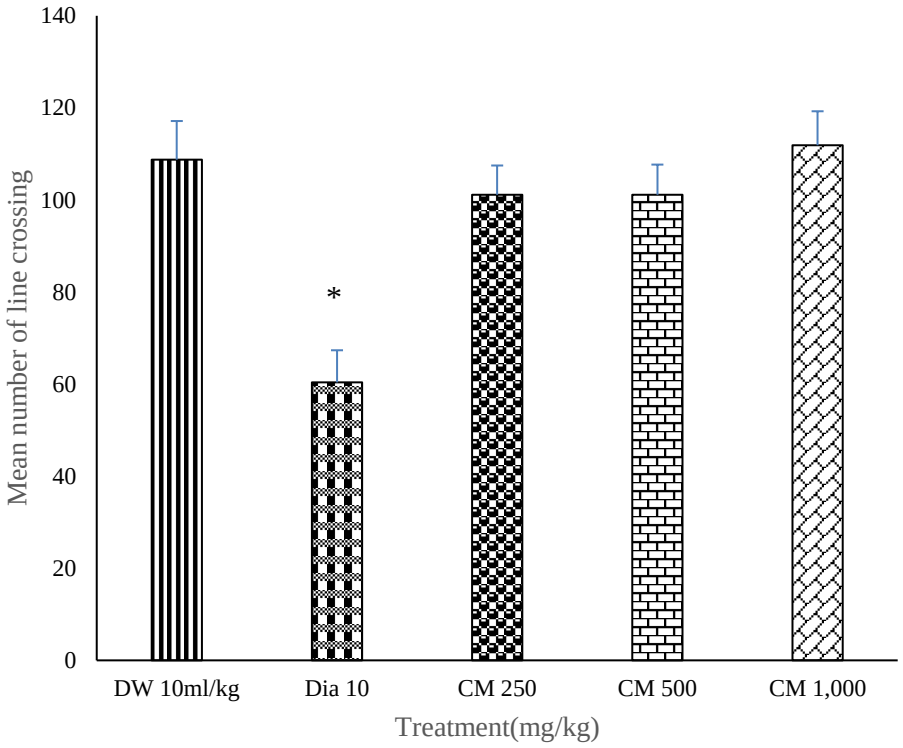


Figure 4.17: **Effect of Methanol Leaf Extract of *Combretum micranthum* on Line Crossing of Mice in the Open Field Test**

Animals were acutely treated with CM (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (10 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.01$ significantly different from distilled water treated animals. CM= *Combretum micranthum*, DW= Distilled water, Dia= Diazepam

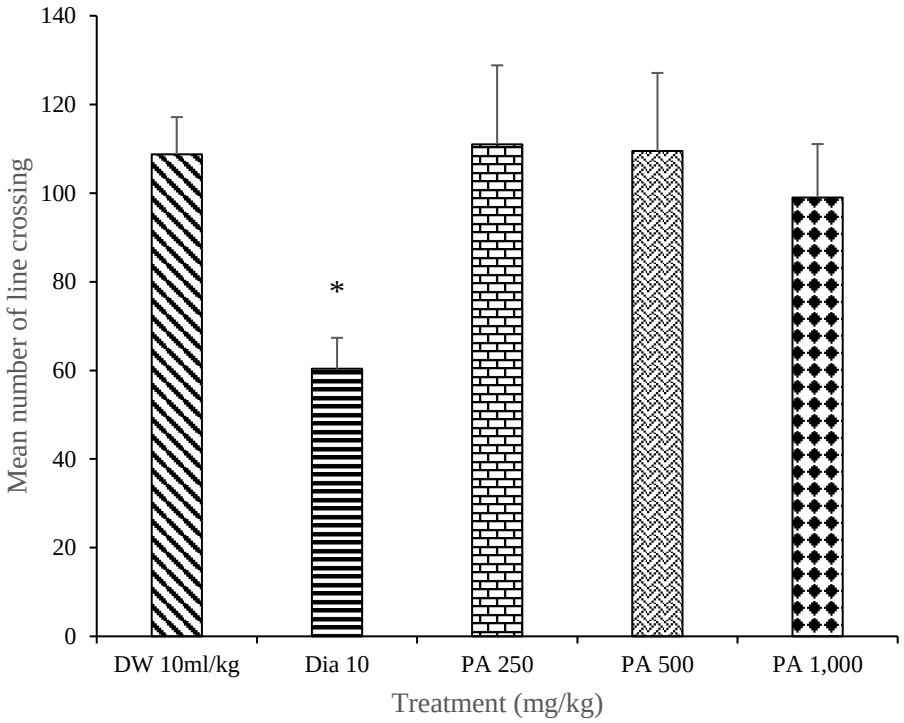


Figure 4.18: **Effect of Methanol Bulb Extract of *Pancrantium africanum* on Line Crossing of Mice in the Open Field Test**

Animals were acutely treated with PA (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or diazepam (10 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.01$ significantly different from distilled water treated animals. PA= *Pancrantium africanum*, DW= Distilled water, Dia= Diazepam

4.5 Antidepressant Activity of Methanol Stem Bark Extrat of *Adansonia digitata*

4.5.1 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Behaviour of Mice in the Forced Swim Test

The methanol stem bark extract of *Adansonia digitata* (250, 500 and 1,000 mg/kg) significantly ($p \leq 0.001$) and dose dependently decreased the duration of immobility as compared to the distilled water-treated group. So also, imipramine (15 mg/kg) significantly decreased the duration of immobility but its effect was not as remarkable as that of the extract (Figure 4.19). Similarly, the methanol stem bark extract of *Adansonia digitata* significantly ($p \leq 0.001$) increased swimming time but not the climbing time of mice subjected to forced swimming test in a dose-dependent manner as compared to the distilled water (10 ml/kg) group. On the other hand, the standard drug imipramine (15 mg/kg) significantly ($p \leq 0.001$) increased climbing time without modification of swimming time (Figure 4.20).

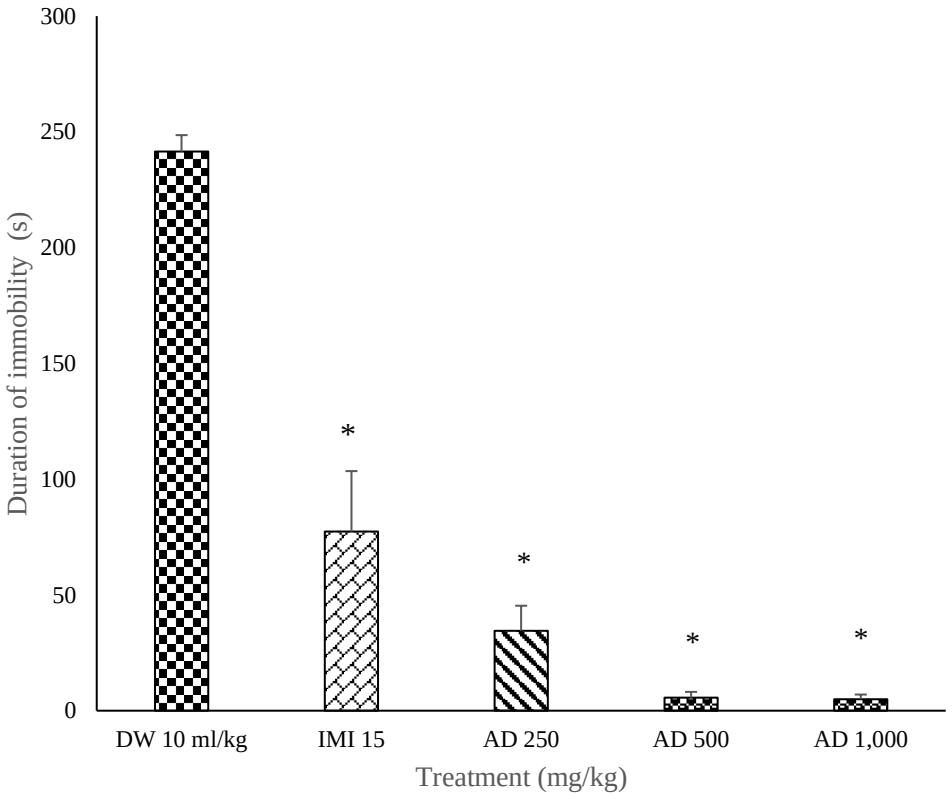


Figure 4.19: **Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Immobility of Mice in the Forced Swim Test**

Animals were acutely treated with AD (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.001$ significantly different from distilled water treated group. IMI= Imipramine, AD= *Adansonia digitata*, DW= Distilled water

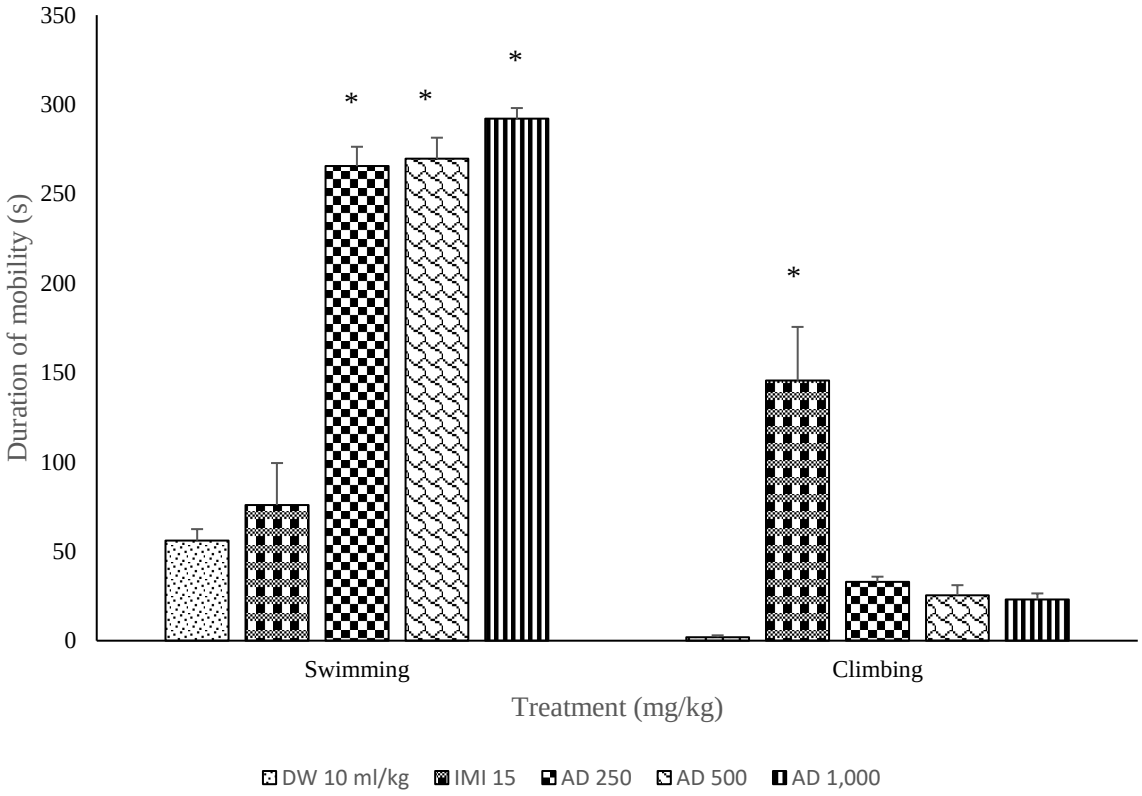


Figure 4.20: **Effect of *Adansonia digitata* on Swimming and Climbing Behaviour in Forced Swim Test in Mice**

Animals were acutely treated with AD (250, 500, or 1,000 mg/kg, *po*), distilled water (10 ml/kg, *po*), or imipramine (15 mg/kg, *po*). Each column represents the mean ± SEM of 8 animals. Data was analysed using one-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.05$ significantly different from distilled water treated group. Imip= Imipramine, AD= *Adansonia digitata*, DW= Distilled water

4.5.2 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Cognitive Behaviour of Mice in the Novel Object Recognition Task

The methanol stem bark extract of *Adansonia digitata* (250, 500 and 1,000 mg/kg) did not significantly alter exploration time of mice in the novel object recognition task (NORT) in the acute phase. Conversely, imipramine (15 mg/kg) significantly decreased the exploration time in the NORT (Figure 4.21).

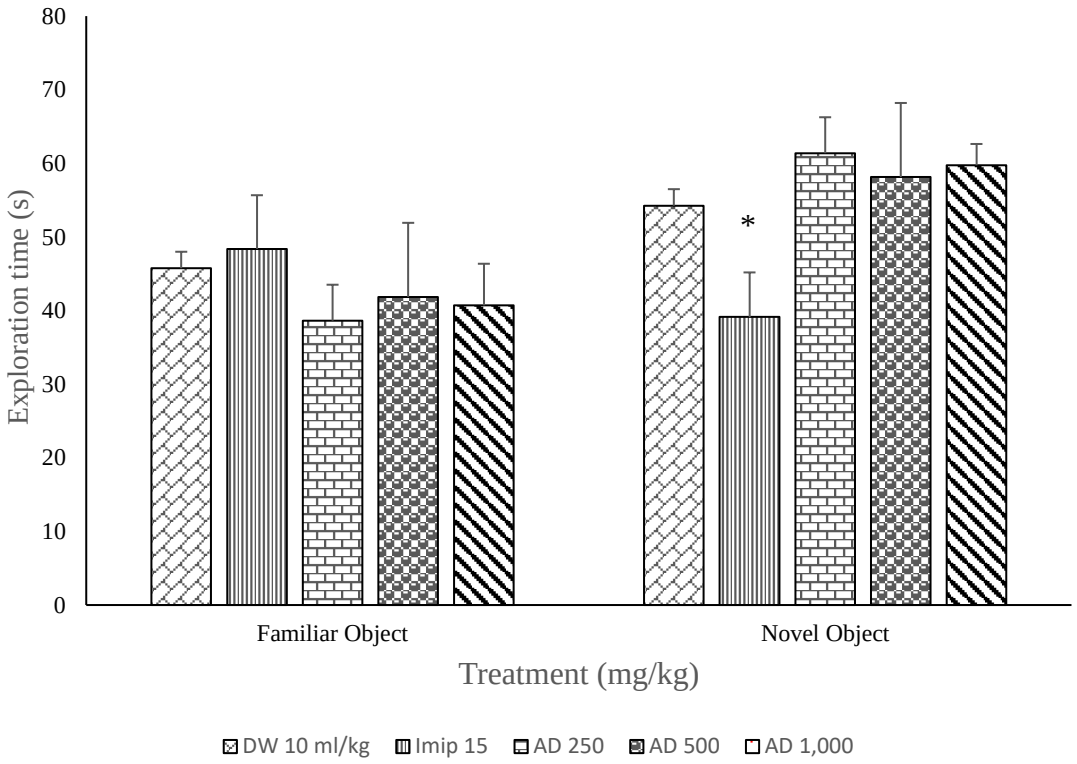


Fig. 4.21: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Exploration Time of Identical and Novel Objects in the Novel Object Recognition Task in Mice

Data expressed as mean±SEM (n=8) and were analysed by one-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.05$ significantly different from distilled water treated group. DW= Distilled water (10 ml/kg, *po*); IMI= Imipramine (15 mg/kg, *po*); AD= methanol stem bark extract of *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*)

4.6 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Chronic Unpredictable Mild Stress-Induced Depression in Mice

4.6.1 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Body Weight of Mice in the Chronic Unpredictable Mild Stress-Induced Depression

There were no significant differences between the body weights of the mice in the six groups at the beginning of the experiment ($p > 0.05$). Thereafter, the mice subjected to stress showed a significant ($p \leq 0.01$) decrease in body weight compared to the unstressed mice from weeks 1 to 5 of the experiment. However, following treatment with MEAD (1,000, 500 and 250 mg/kg) and imipramine 15 mg/kg, there was significant ($p \leq 0.01$) increase in body weight as compared to the stressed only group at the fourth and fifth week of the experiment (Figure 4.22).

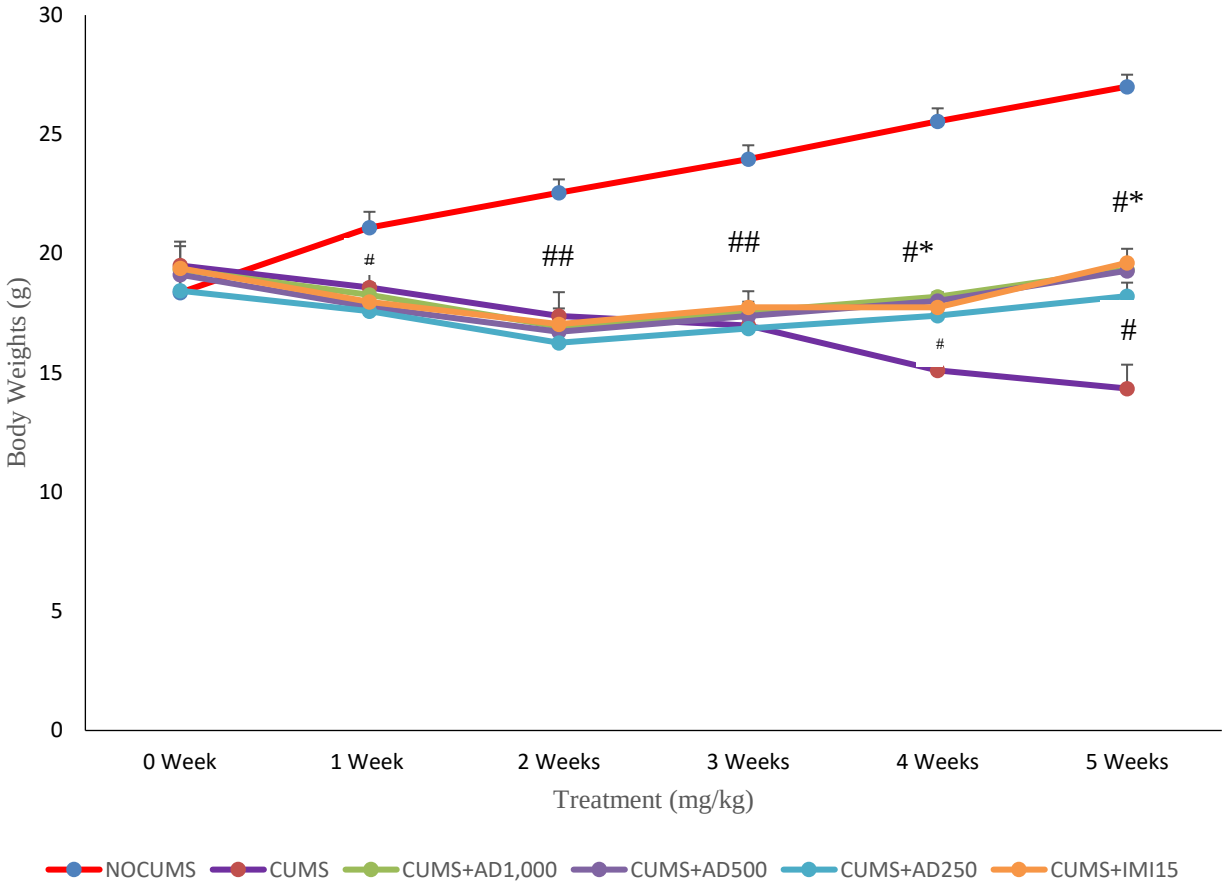


Figure 4.22: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Body Weight of Mice following Chronic Unpredictable Mild Stress

The body weights following CUMS in mice were recorded six times, once every week. Data were analysed using Repeated measures ANOVA followed by Bonferoni post hoc test, presented as mean ± SEM (n = 8 - 12). $^{\#}p \leq 0.01$ & $^{\#\#}p \leq 0.001$ significant difference as compared to NOCUMS group; $^{*}p \leq 0.05$ significant difference as compared to CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*)

4.6.2 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Sucrose Consumption following Chronic Unpredictable Mild Stress in Mice

At the beginning of the experiment, there was no significant difference in the sucrose consumption across the groups. However, two weeks after chronic unpredictable mild stress exposure, the sucrose consumption significantly ($p \leq 0.05$) decreased in all the groups except in the unstressed group mice. Additionally, three weeks after treatment with methanol stem bark extract of *Adansonia digitata* (250, 500 and 1,000 mg/kg) and imipramine (15 mg/kg), there was a significant ($p \leq 0.05$, $p \leq 0.01$, $p \leq 0.001$) increase in sucrose consumption in mice from all the groups as compared to the stressed group (Figure 4.23).

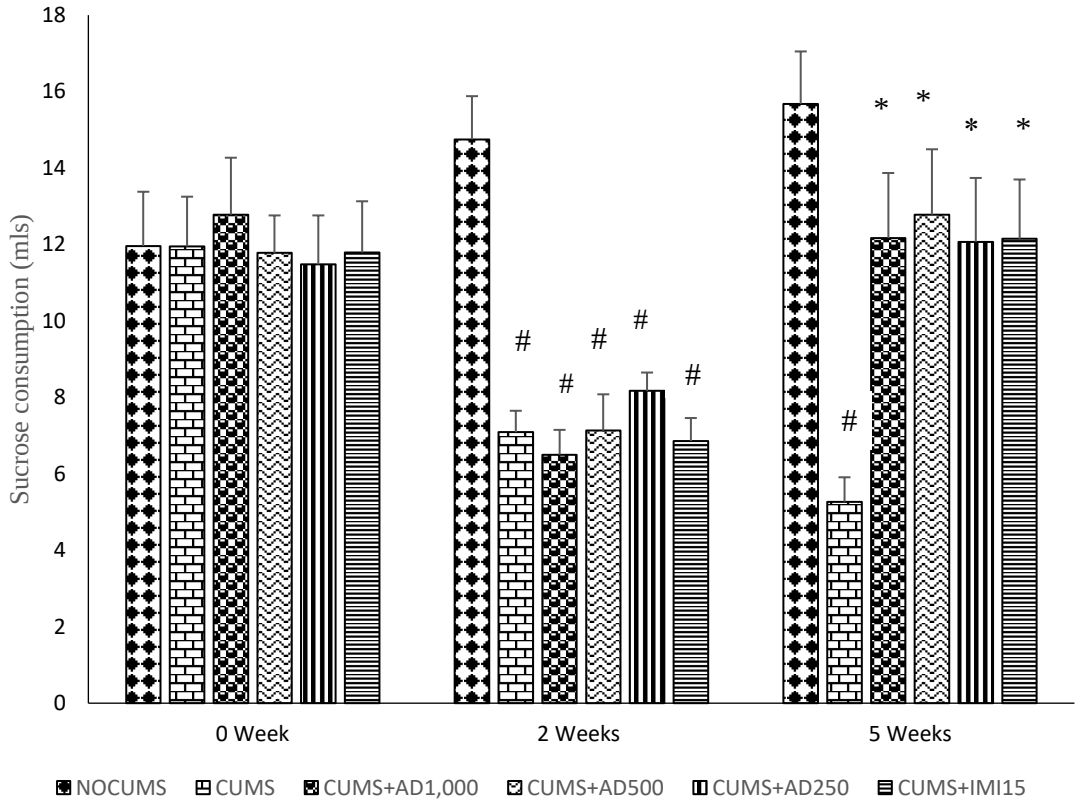


Figure 4.23: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Sucrose Consumption of Mice following Chronic Unpredictable Mild Stress

Results were represented as mean ± SEM (n = 8-12). Data were analysed using one-way ANOVA followed by Bonferroni post hoc test, $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$ significant difference as compared to NOCUMS group; $^{*}p \leq 0.001$ significant difference as compared to CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*)

4.6.3 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Locomotory Behaviour in Mice following Chronic Unpredictable Mild Stress-Induced Depression

At week 0, there was no significant difference in locomotor activity prior to subjecting the mice to stress. There was significant decrease in number of line crossing ($p \leq 0.001$) in the mice subjected to the chronic unpredictable mild stress for two consecutive weeks without treatment. However, increased line crossing ($p \leq 0.001$) were exhibited by the mice treated with methanol extract of *Adansonia digitata* (250, 500 and 1,000 mg/kg) for three weeks as compared to the stressed mice. The group treated with imipramine (15 mg/kg) showed no increase in the number of line crossed when compared to the stressed group (Figure 4. 24).

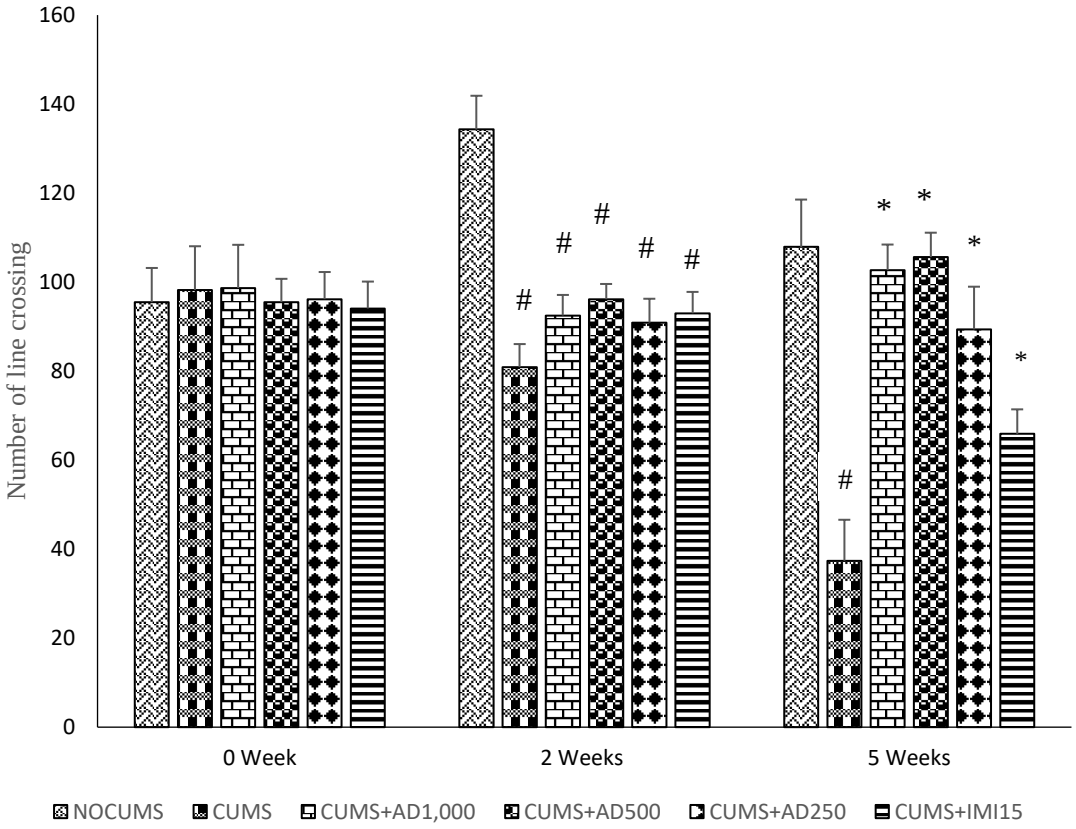


Figure 4.24: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Line Crossing Behaviour of Mice following Chronic Unpredictable Mild Stress

Data were analysed using one-way ANOVA followed by Bonferoni post hoc test, expressed as mean ± SEM (n = 8-12). $^{\#}p<0.05$ significant difference as compared to NOCUMS group, $*p\leq 0.05$, $**p\leq 0.01$ significant difference as compared with CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*)

4.6.4 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Duration of Immobility in the Chronic Unpredictable Mild Stress-Induced Depression in Mice

There were no significant differences in the duration of immobility in all the groups at the commencement of the study (week 0). However, 2 weeks after subjecting mice to stressors, the duration of immobility significantly ($p \leq 0.01$) increased in all the groups when compared to the unstressed group. Treatment with MEAD (250, 500 and 1,000 mg/kg) and imipramine (15 mg/kg) significantly ($p \leq 0.01$) and dose dependently decreased the duration of immobility when compared to the stressed mice (Figure 4.25).

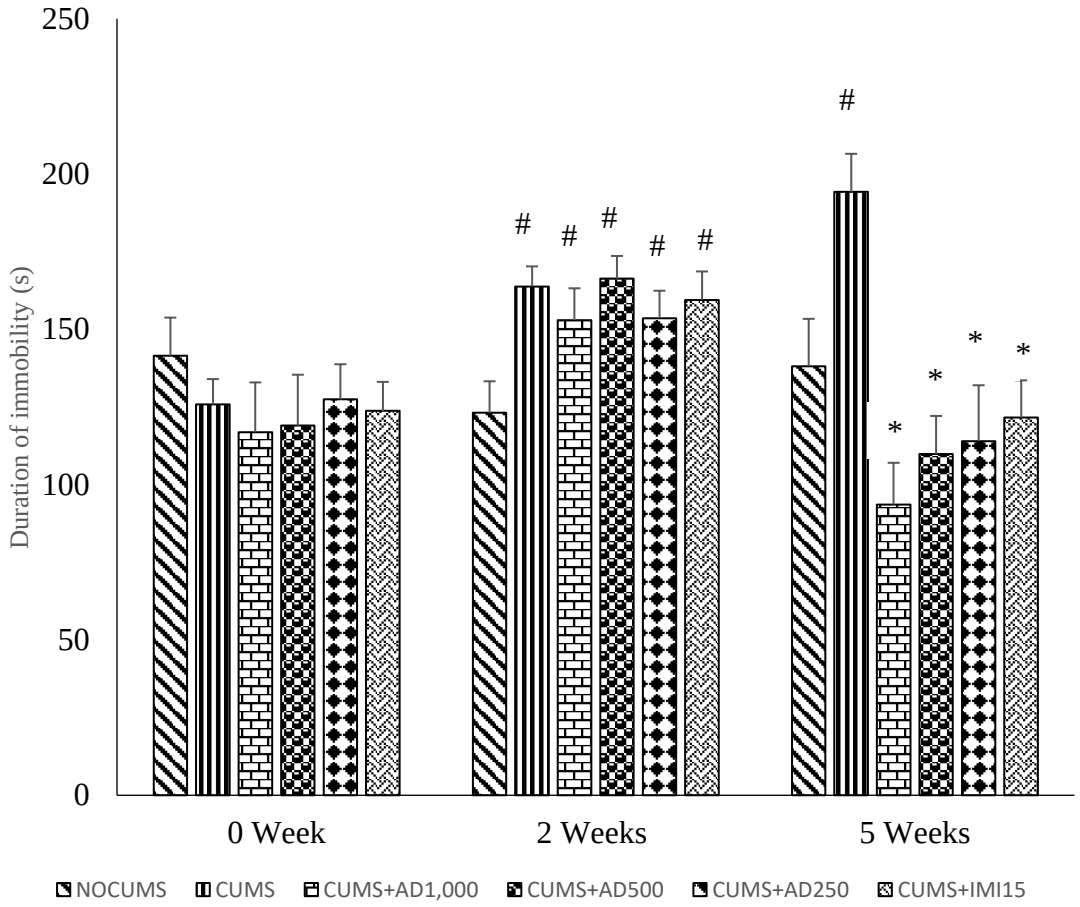


Figure 4.25: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Duration of Immobility in Mice following Chronic Unpredictable Mild Stress

All results were expressed as mean ± SEM (n = 8-12). Data were analysed using one-way ANOVA followed by Bonferoni post hoc test, #p < 0.05 significant difference as compared to NOCUMS group; **$p\leq$ 0.01, ***$p\leq$ 0.001 significant difference as compared to CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1, 000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*)

4.6.5 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Exploratory Behaviour in the Chronic Unpredictable Mild Stress-Induced Depression in Mice

From this study, there was significant ($p \leq 0.001$) increase and a significant ($p \leq 0.001$) decrease in exploration time of familiar and novel object respectively in the stressed mice when compared to the unstressed mice. However, treatment with MEAD (250, 500 and 1,000 mg/kg) and imipramine (15 mg/kg) significantly ($p \leq 0.01$) increased the exploration time of the novel object as compared to the stressed group (Figure 4.26).

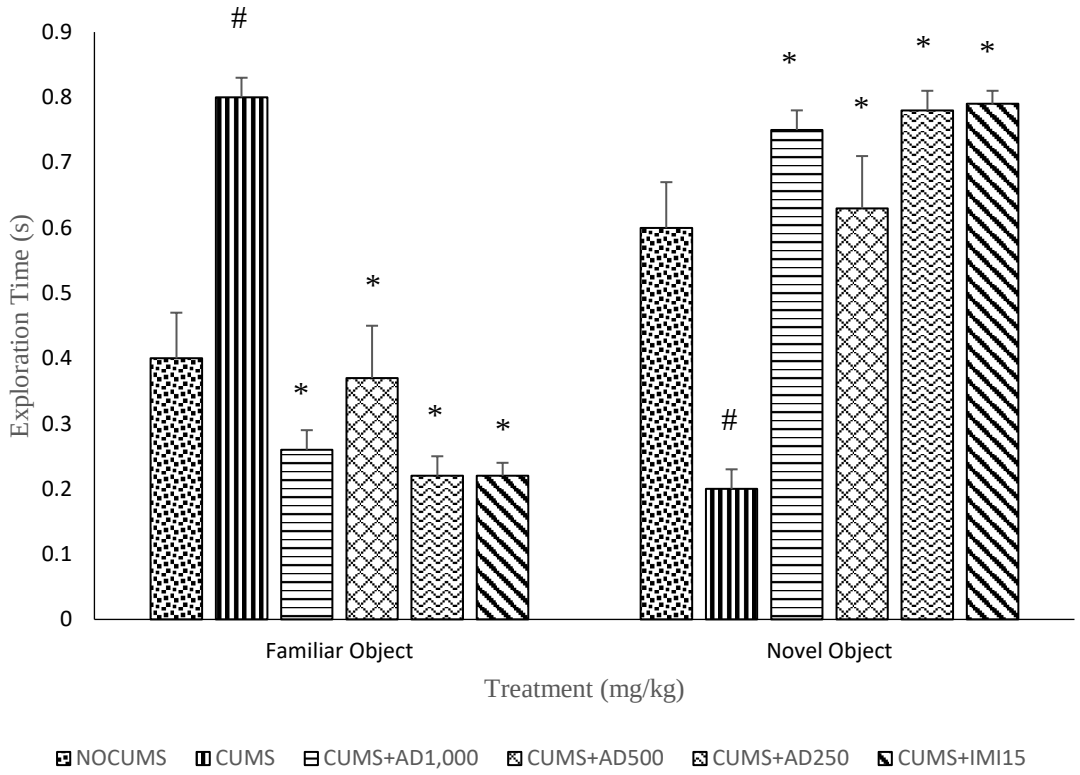


Figure 4.26: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Novel Object Exploration Time in Mice following Chronic Unpredictable Mild Stress

Data were presented as mean ± SEM (n = 8). Data were analysed using one-way ANOVA followed by Bonferoni post hoc test, #$p \leq$ 0.001 significant difference as compared to NOCUMS group; *$p \leq$ 0.001 significant difference as compared to CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*)

4.6.6 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Plasma Cortisol level in the Chronic Unpredictable Mild Stress-Induced Depression in Mice

The plasma levels of cortisol were significantly ($p \leq 0.01$) increased in the stressed mice when compared to the unstressed mice. However, treatment with MEAD (250, 500 and 1,000 mg/kg) and imipramine (15 mg/kg) significantly ($p \leq 0.01$) and dose dependently reduced the levels of plasma cortisol when compared to the stressed mice (Figure 4.27).

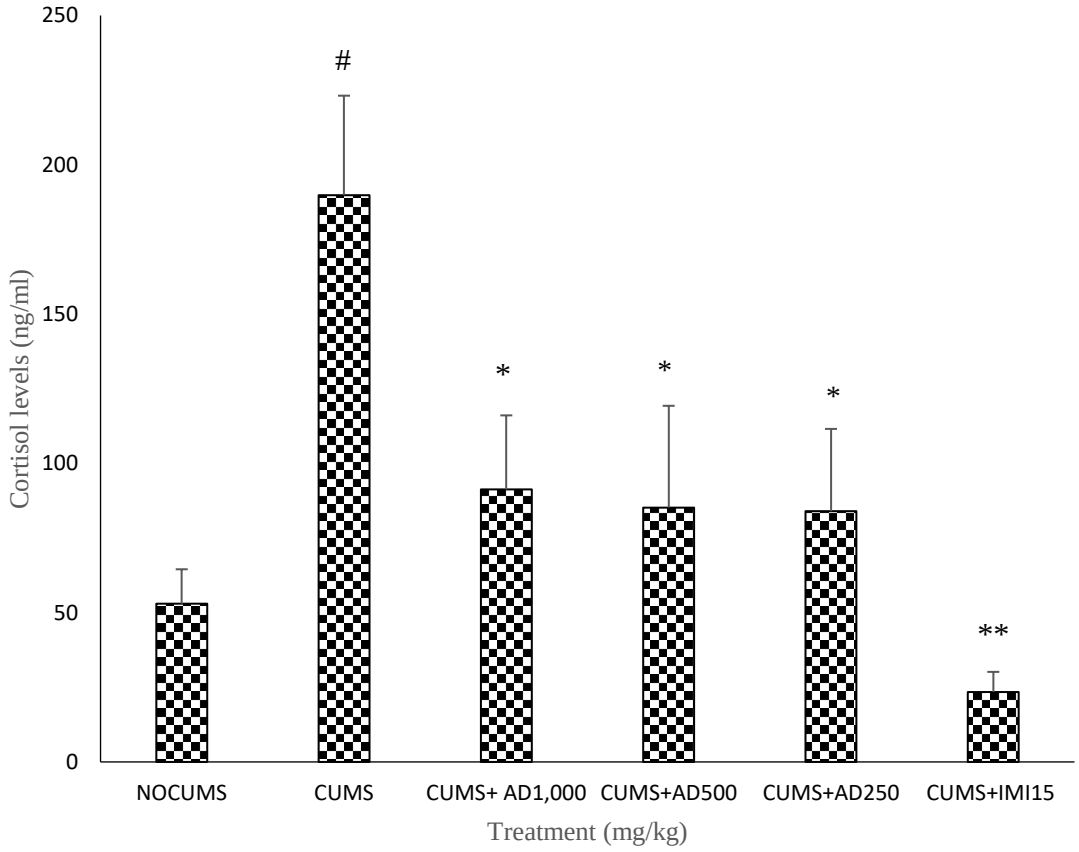


Figure 4.27: Effect of Methanol Stem Bark extract of *Adansonia digitata* on Plasma Cortisol levels in Mice following Chronic Unpredictable Mild Stress

All results were expressed as mean ± SEM (n = 8). Data were analysed using one-way ANOVA followed by Bonferoni post hoc test, $^{\#}p \leq 0.01$ significant difference as compared to NOCUMS group; $*p \leq 0.05$, $**p \leq 0.01$ significant difference as compared to CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*)

4.6.7 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Brain Derived Neurotrophic Factor Levels in Mice

As shown in Figure 4.37, brain derived neurotrophic factor (BDNF) levels were measured at the end of the experiment. The level of BDNF protein concentration in the brain was significantly ($p \leq 0.01$) lowered in the mice subjected to 5 weeks of the chronic unpredictable mild stress procedure when compared with the unstressed mice. Additionally, treatment with MEAD (250, 500 and 1,000 mg/kg) and imipramine (15 mg/kg) markedly increased the level of BDNF protein expression in the brain ($p \leq 0.05$, $p \leq 0.001$) when compared to the stressed group. Treatment with MEAD 1,000 mg/kg reversed BDNF to normal levels (Figure 4.28).

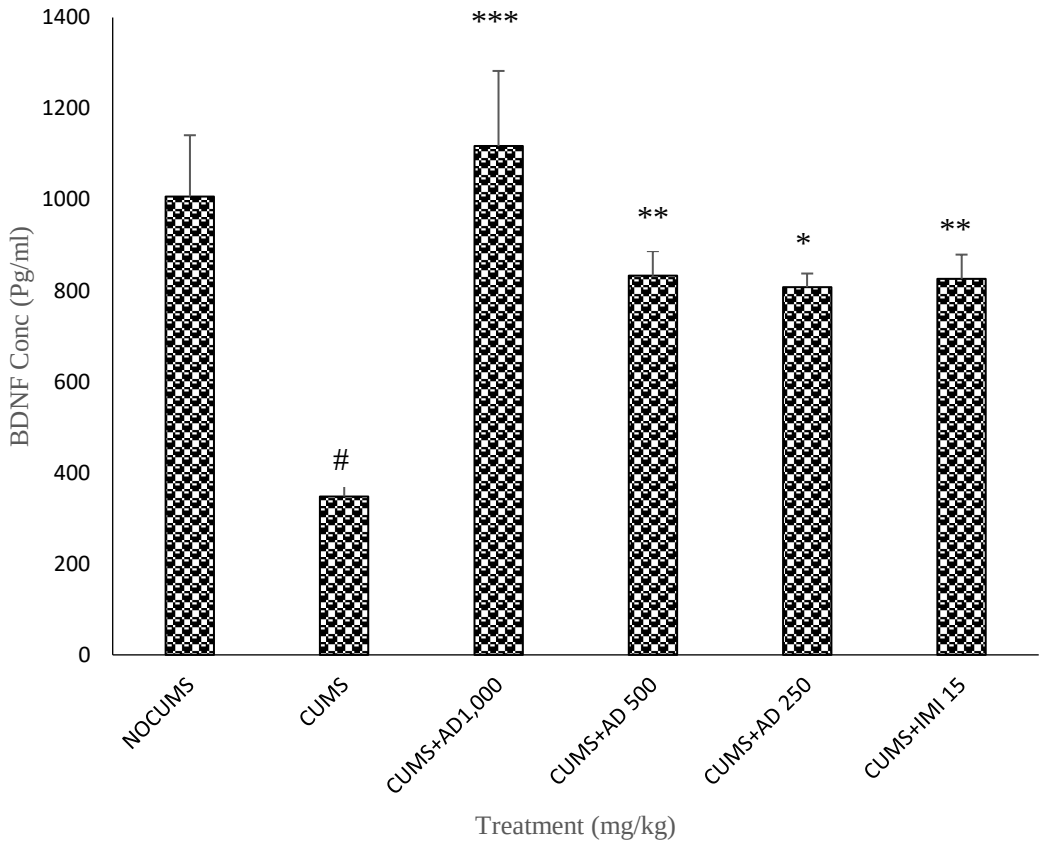


Figure 4.28: Effect of Methanol Stem Bark extract of *Adansonia digitata* on BDNF Concentration in Mice following Chronic Unpredictable Mild Stress

All results were expressed as mean ± SEM (n = 8). $^{\#}p \leq 0.01$ significant difference as compared to NOCUMS group; $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$ significant difference as compared to CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*), Conc= Concentration, BDNF= Brain Derived Neurotrophic Factor

4.6.8 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Plasma Malondialdehyde levels in Chronic Unpredictable Mild Stress-Induced Depression in Mice

From the result, there was a significant ($p \leq 0.05$) increase in plasma MDA levels in mice subjected to chronic unpredictable mild stress as compared to the unstressed group. However, treatment with MEAD (250, 500 and 1,000 mg/kg) significantly ($p \leq 0.01$ & $p \leq 0.001$) and dose dependently decreased plasma MDA levels when compared to the stressed group. Unlike MEAD, imipramine (15 mg/kg) did not significantly decrease plasma MDA levels when compared to the stressed group (Figure 4.29).

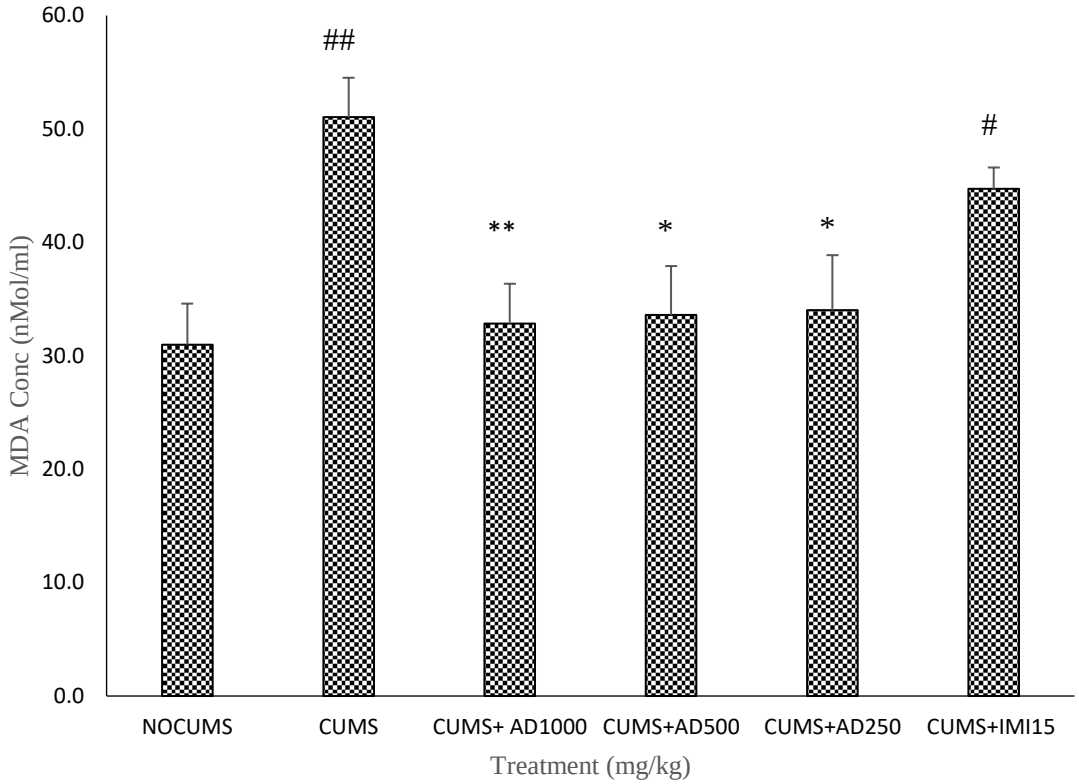


Figure 4.29: Effect of Methanol Stem Bark extract of *Adansonia digitata* on Plasma Malondialdehyde levels in Mice following Chronic Unpredictable Mild Stress

All results were expressed as mean ± SEM (n = 8). $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$ significant difference as compared to Unstressed group; $^{*}p \leq 0.01$, $^{**}p \leq 0.001$ significant difference as compared to Stressed group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*), Conc= Concentration, MDA= Malondialdehyde

4.6.9 Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Total Erythrocyte Superoxide Dismutase Concentration in Chronic Unpredictable Mild Stress-Induced Depression in Mice

The erythrocyte total SOD activity was measured at the end of the experiment. Exposure to stressor for 5 weeks caused a significant ($p \leq 0.01$) depletion in the concentration of erythrocyte SOD in stressed mice when compared with the unstressed mice. However, there was significant ($p \leq 0.05$) and dose dependent increase in the concentration of SOD following treatment with methanol extract of *A. digitata* as compared to the stressed group. The imipramine 15 mg/kg did not significantly increase the level of erythrocyte SOD activity as compared to the stressed group (Figure 4.30).

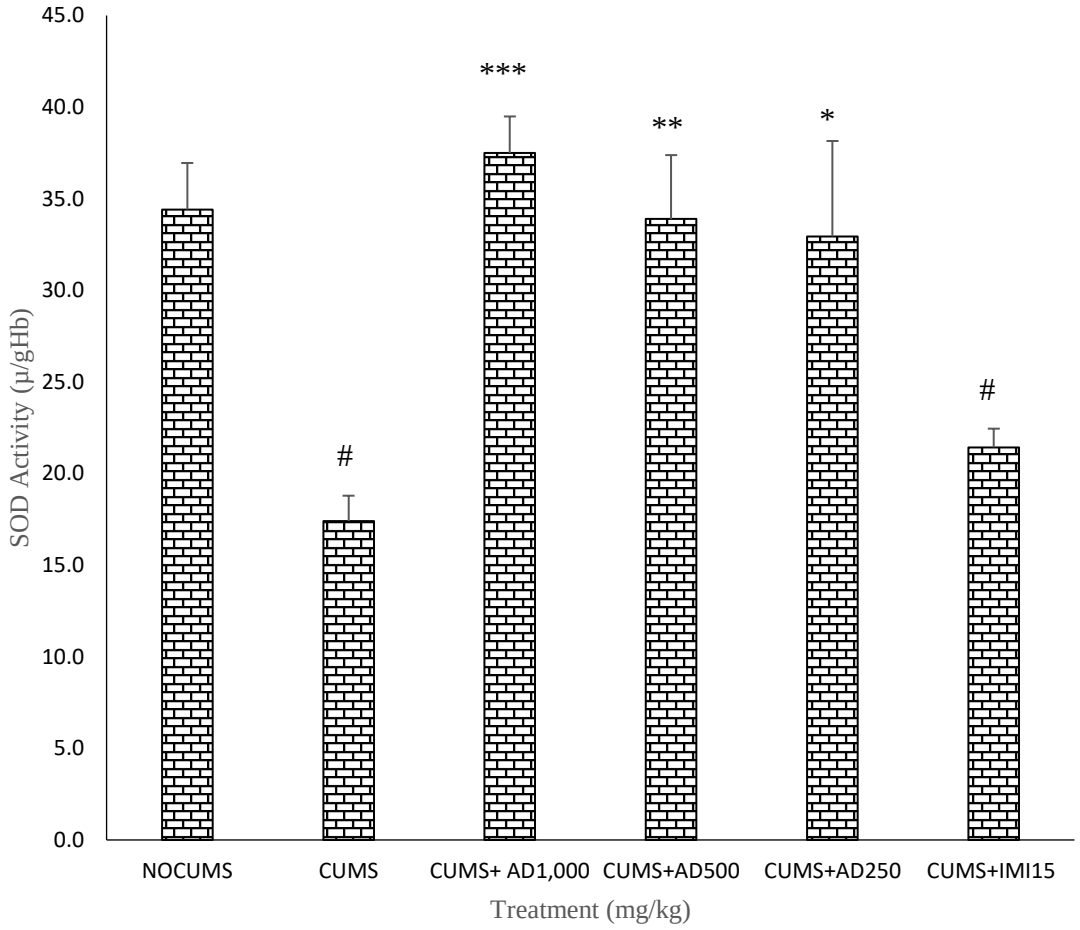


Figure 4.30: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Erythrocyte Total Superoxide Dismutase activity in Mice following Chronic Unpredictable Mild Stress

All results were expressed as mean ± SEM (n = 8). $^{\#}p \leq 0.01$ significant difference as compared to Unstressed group; $^{*}p \leq 0.05$, $^{**}p \leq 0.01$, $^{***}p \leq 0.001$ significant difference as compared to Stressed group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*), Conc= Concentration, SOD= Superoxide Dismutase

4.7 Evaluation of Possible Mechanism of Action of Methanol Stem Bark Extract of *Adansonia digitata*

4.7.1 Involvement of Nitric Oxide Pathway

Methanol stem bark extract of *Adansonia digitata* (1,000 mg/kg) and imipramine (15 mg/kg) significantly ($p \leq 0.01$) decreased duration of immobility as compared to the distilled water treated group. The decreased duration of immobility by both extract and imipramine was not reversed by the L-Arginine (Figure 4.31). Pre-treatment with L-NNA significantly ($p \leq 0.01$) decreased duration of immobility than that observed with methanol stem bark extract of *A. digitata* alone. As compared to the distilled water group, L-NNA (nitric oxide synthase enzyme inhibitor) pretreated group showed greater decrease in duration of immobility than the extract alone (Figure 4.32).

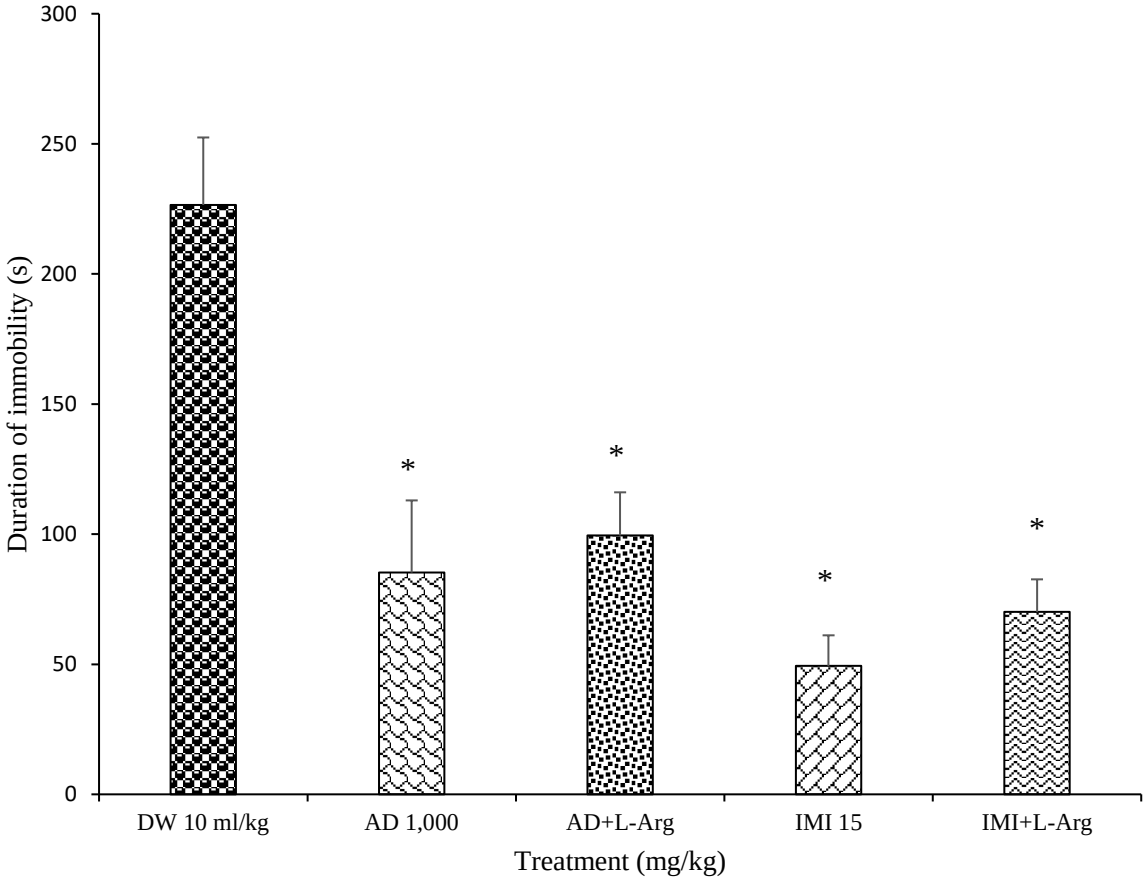


Figure 4.31: **Effect of L-Arginine on Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* in the Mouse Tail Suspension Test**

Each column represents the mean ± SEM, n= 8. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.01$, significantly different from DW group; AD= *Adansonia digitata* (1,000 mg/kg, *po*); IMI= Imipramine (15 mg/kg, *po*); L-Arg= L-Arginine (50 mg/kg, *ip*); DW= Distilled water

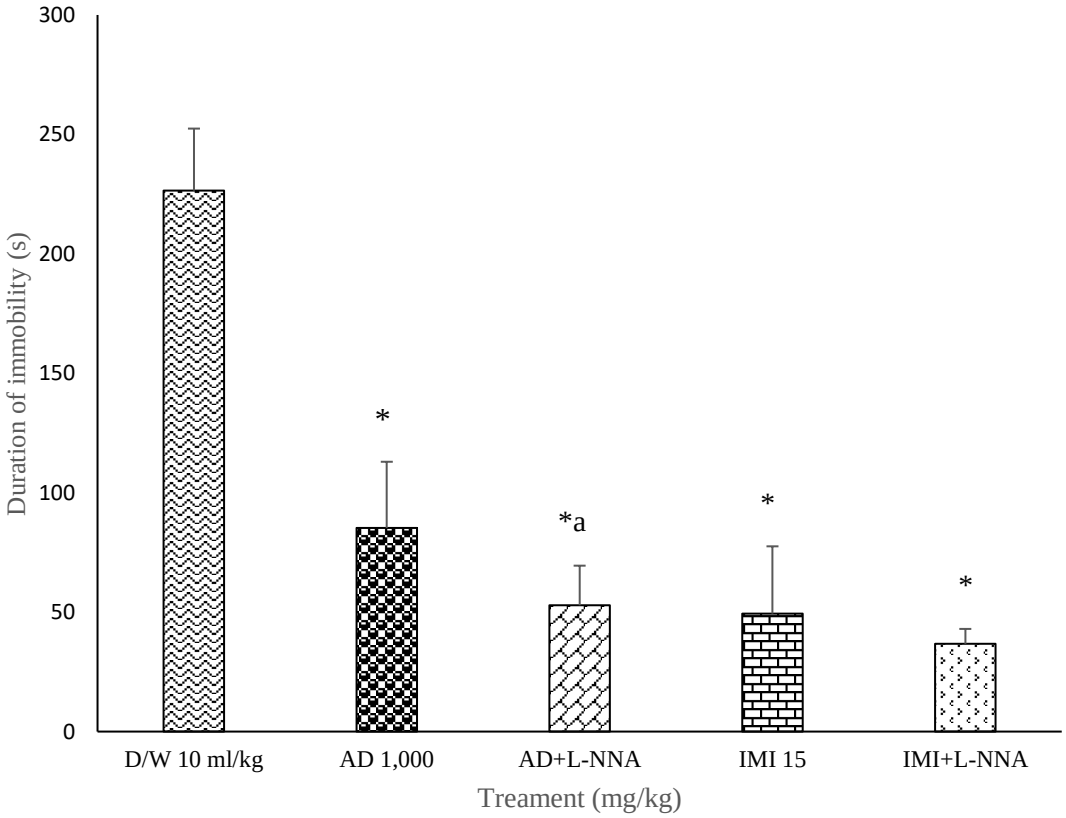


Figure 4.32: **Effect of L-NNA on Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* in the Mouse Tail Suspension Test**

Each column represents the mean ± SEM, n= 8. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.01$, significantly different from DW group; $^{a}p \leq 0.01$ significantly different from AD-treated group; AD= *Adansonia digitata* (1,000 mg/kg, *po*); IMI= Imipramine (15 mg/kg, *po*); L-NNA= L-Nitro N-Arginine (50 mg/kg, *ip*); DW= Distilled water

4.7.2 Involvement of Dopaminergic System

The methanol extract of *A. digitata* (1,000 mg/kg) and imipramine (15 mg/kg) significantly ($p \leq 0.01$) reduced the duration of immobility as compared to distilled water treated group. However, sulpiride significantly ($p \leq 0.01$) increased the duration of immobility of extract and/or imipramine when co-administered (Figure 4.33).

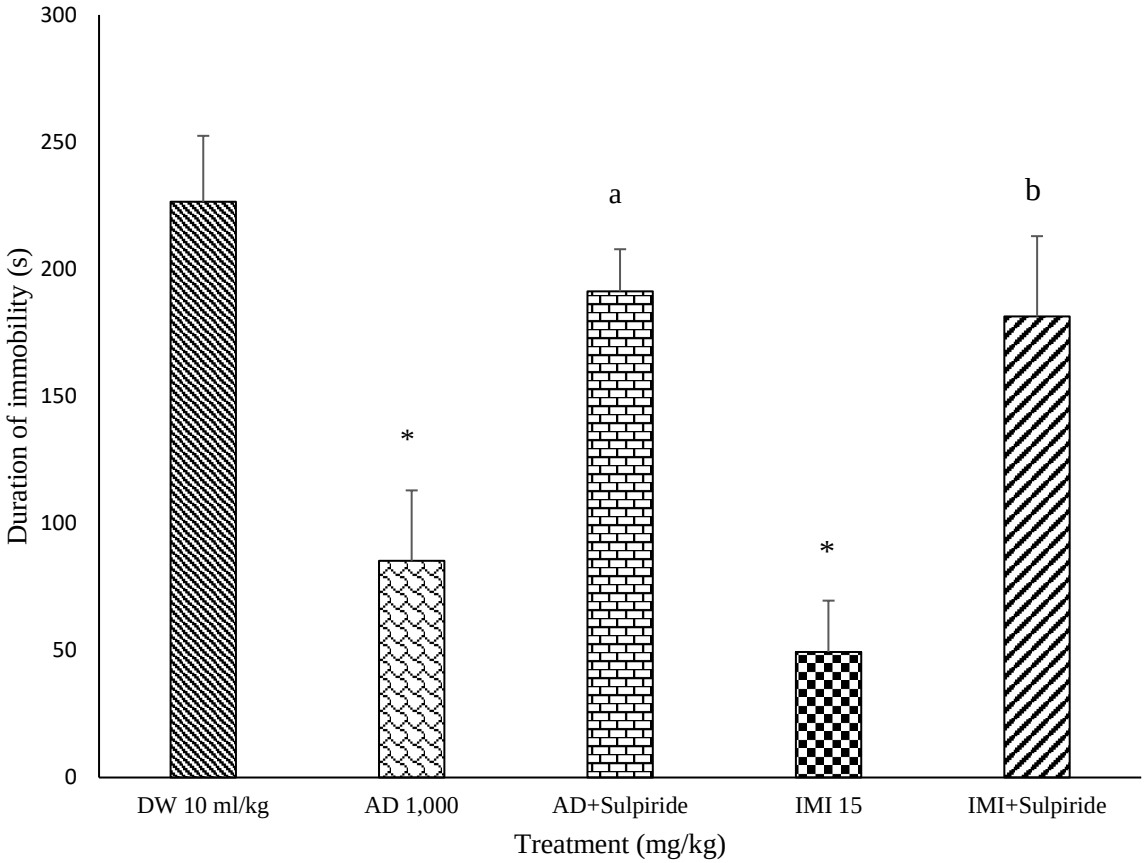


Figure 4.33: **Effect of Sulpiride on Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* in the Mouse Tail Suspension Test**

Each column represents the mean ± SEM, n= 8. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.001$ significantly different from DW treated group; a & b= $p \leq 0.01$ significantly different from AD and IMI treated groups respectively; AD= *Adansonia digitata* (1,000 mg/kg, *po*); IMI= Imipramine (15 mg/kg, *po*); DW= Distilled water (10 ml/kg, *po*), Sulpiride (50 mg/kg, *ip*)

4.7.3 Involvement of Noradrenergic System

Pretreatment with prazosin (α_1 adrenergic antagonist) insignificantly increased the duration of immobility of mice treated with methanol stem bark extract of *Adansonia digitata* (1,000 mg/kg). However, the effect of imipramine (15 mg/kg) was not affected (Figure 4.34). Pretreatment with yohimbine (α_2 adrenergic antagonist) significantly ($p \leq 0.01$) increased the duration of immobility of mice treated with imipramine and non significantly increased the duration of immobility of mice treated with methanol stem bark extract of *Adansonia digitata* (Figure 4.35).

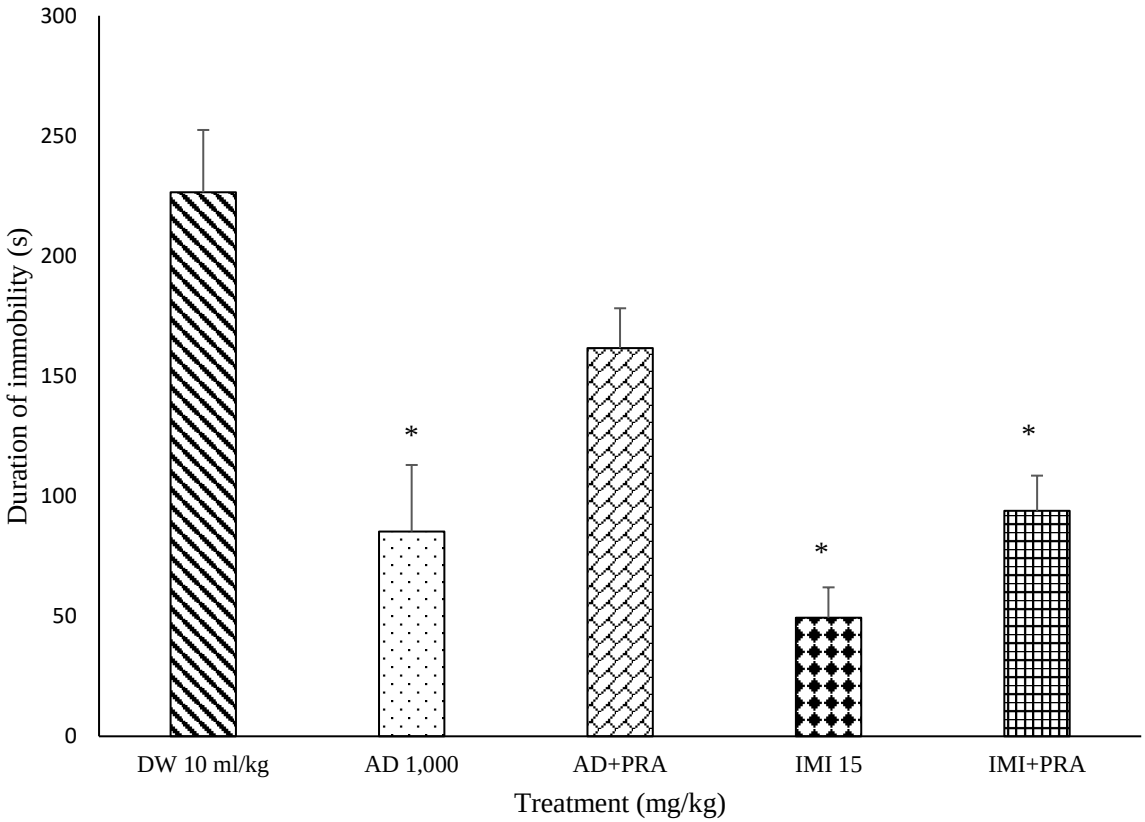


Figure 4.34: **Effect of Prazosin on Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* in the Mouse Tail Suspension Test**

Each column represents the mean ± SEM, n= 8. Data analysis was performed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.01$ significantly different from DW group; AD= *Adansonia digitata* (1,000 mg/kg, *po*); IMI= Imipramine (15 mg/kg); PRA= Prazosin (1 mg/kg), DW= Distilled water (10 ml/kg, *po*)

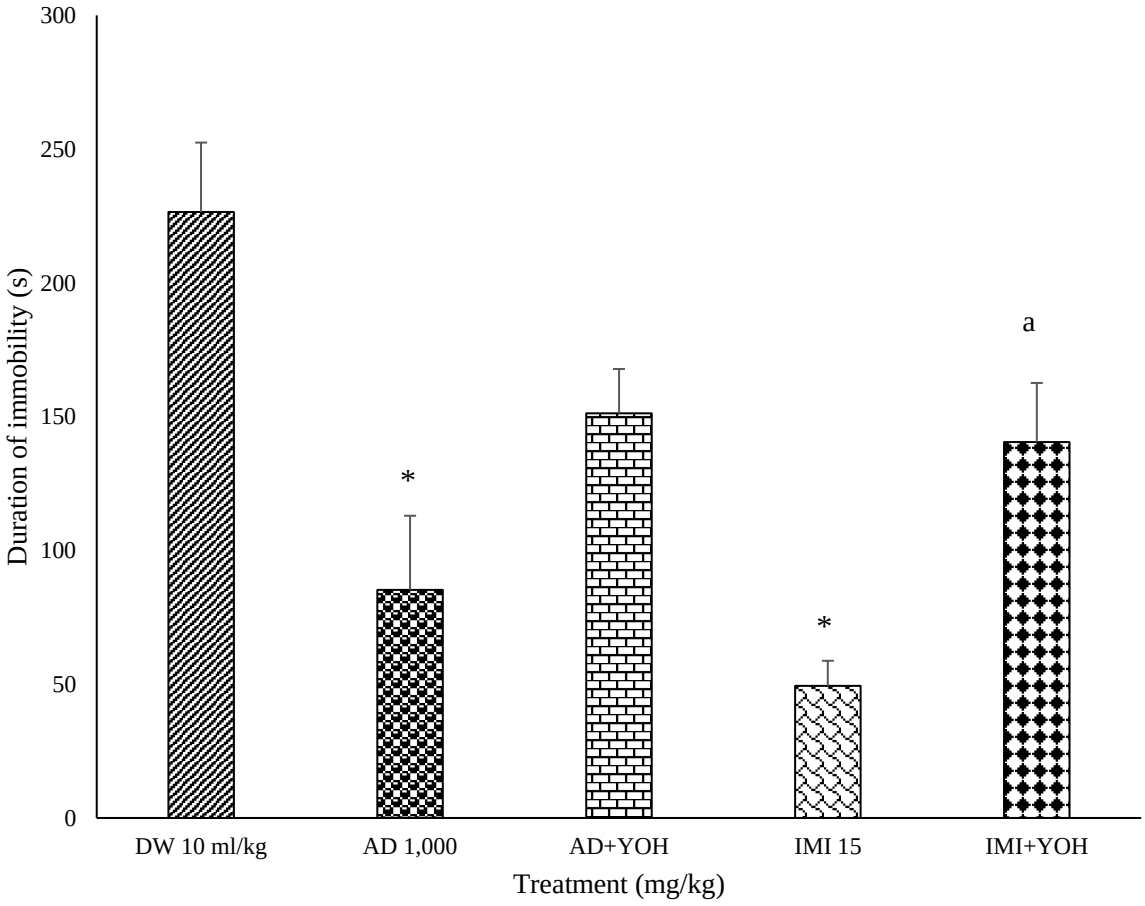


Figure 4.35: **Effect of Yohimbine on Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* in the Mouse Tail Suspension Test**

Each column represents the mean ± SEM, n= 8. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.001$ significantly different from DW treated group; a= $p \leq 0.01$ significantly different from Imipramine treated group; AD= *Adansonia digitata* (1,000 mg/kg, *po*); IMI= Imipramine, DW= Distilled water (10 ml/kg, *po*), YOH= Yohimbine (1 mg/kg, *ip*)

4.7.4 Involvement of Serotonergic System

Metergoline (5-HT_1 receptor antagonist) non significantly increased the duration of immobility of mice treated with methanol stem bark extract of *Adansonia digitata* (1,000 mg/kg) but did not alter duration of immobility of imipramine (15 mg/kg) treated mice (Figure 4.36). On the other hand, cyproheptadine insignificantly increased the duration of immobility of the extract with no effect of the imipramine (Figure 4.37).

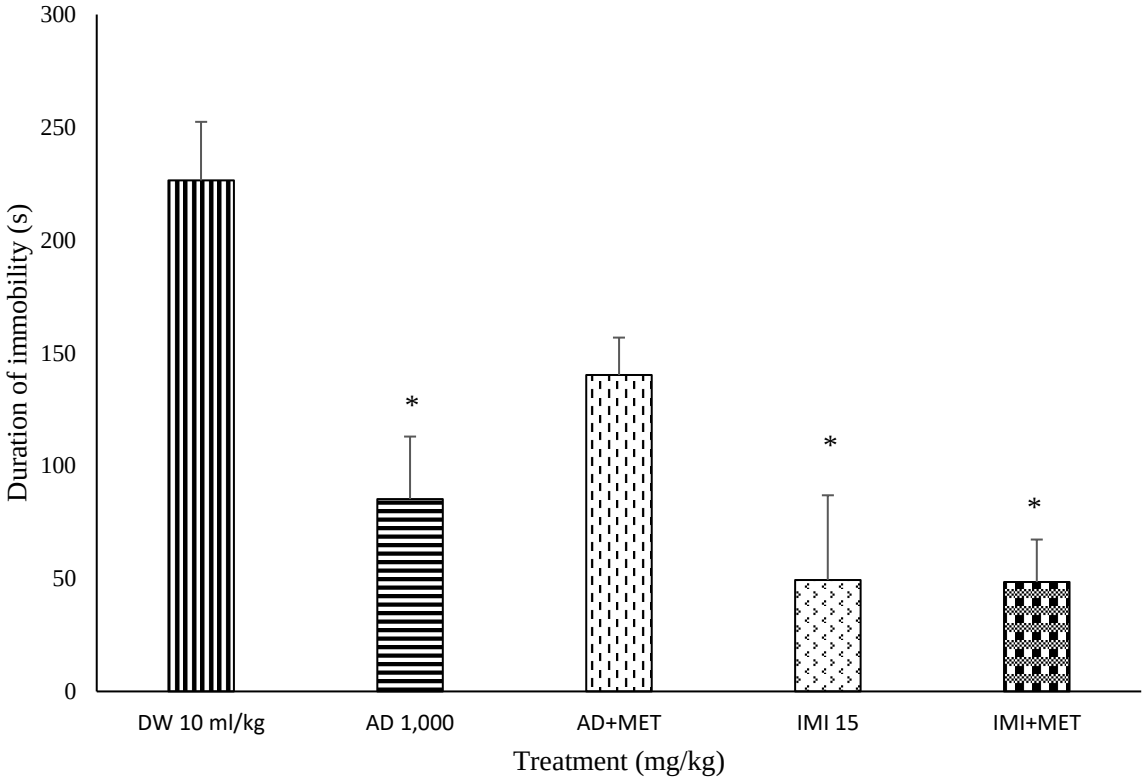


Figure 4.36: **Effect of Metergoline on Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* in the Mouse Tail Suspension Test**

Each column represents the mean ± SEM, n= 8. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.001$ significantly different from DW group; AD= *Adansonia digitata* (1000 mg/kg, *po*); IMI= Imipramine (15 mg/kg, *po*), DW= Distilled water (10 ml/kg, *po*), MET= Metergoline (1 mg/kg, *ip*)

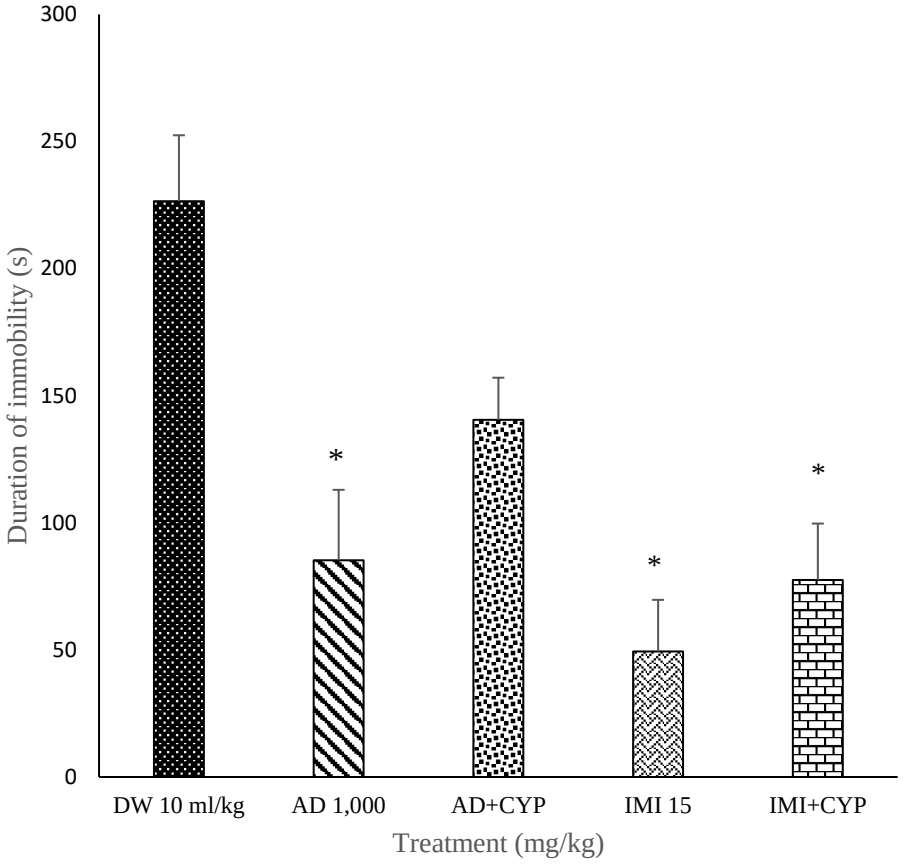


Figure 4.37: **Effect of Cyproheptadine on Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* in the Mouse Tail Suspension Test**

Each column represents the mean ± SEM, n= 8. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.001$ significantly different from DW group; AD= *Adansonia digitata* (1,000 mg/kg, *po*); IMI= Imipramine (15 mg/kg, *po*), DW= Distilled water (10 ml/kg, *po*), CYP= Cyproheptadine (3 mg/kg, *ip*)

4.7.5 Involvement of Cholinergic Pathway

Pretreatment with atropine significantly ($p \leq 0.01$) decreased the duration of immobility of mice treated with methanol stem bark extract of *Adansonia digitata* (1,000 mg/kg). On the other hand, atropine pretreatment did not significantly change the duration of immobility of mice treated with imipramine (Figure 4.38).

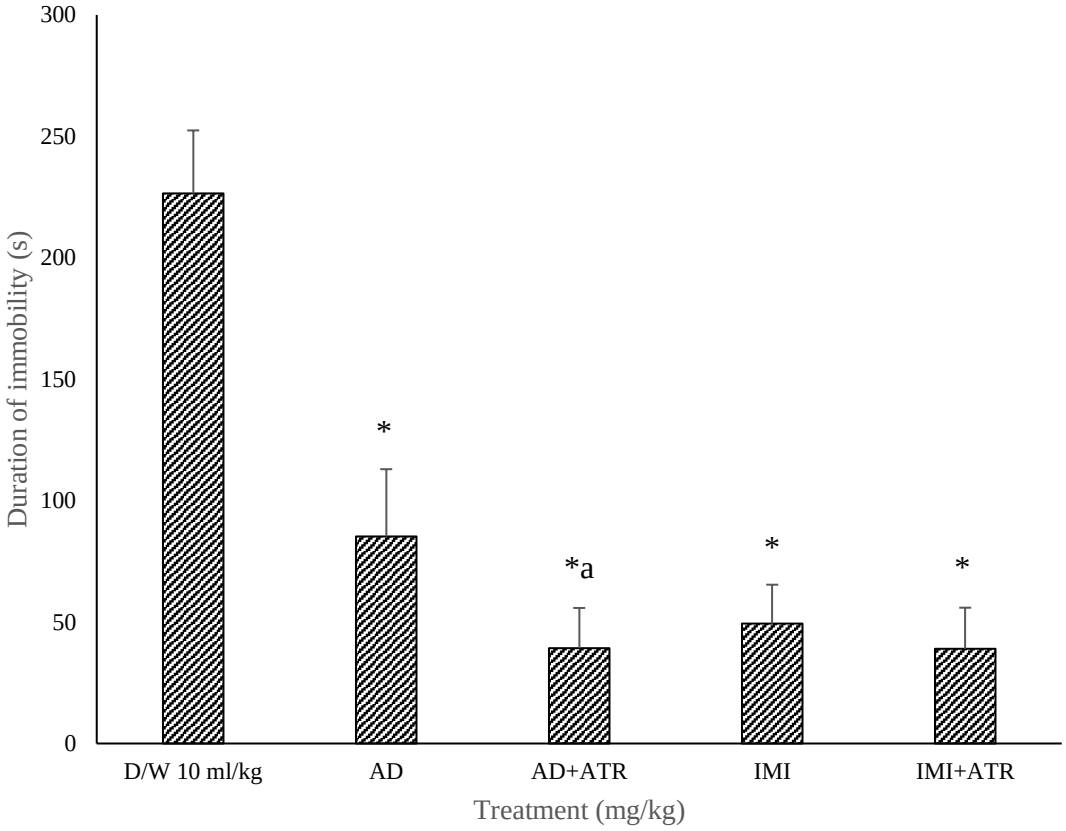


Figure 4.38: **Effect of Atropine on Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* in the Mouse Tail Suspension Test**

Data are mean ± SEM, n= 8, analysed using One-way ANOVA followed by Bonferroni post hoc test, *= $p \leq 0.001$ significantly different from DW group; $^{a}p \leq 0.001$ significantly different from AD treated group; AD= *Adansonia digitata* (1, 000 mg/kg, *po*); IMI= Imipramine (15 mg/kg, *po*), DW= Distilled water (10 ml/kg, *po*), ATR= Atropine (1 mg/kg, *ip*)

4.7.6 Involvement of Opioid Pathways

Pretreatment with naloxone nonsignificantly decreased the duration of immobility of mice treated with methanol stem bark extract of *Adansonia digitata* (1,000 mg/kg) and/or imipramine 15 mg/kg (Figure 4.39).

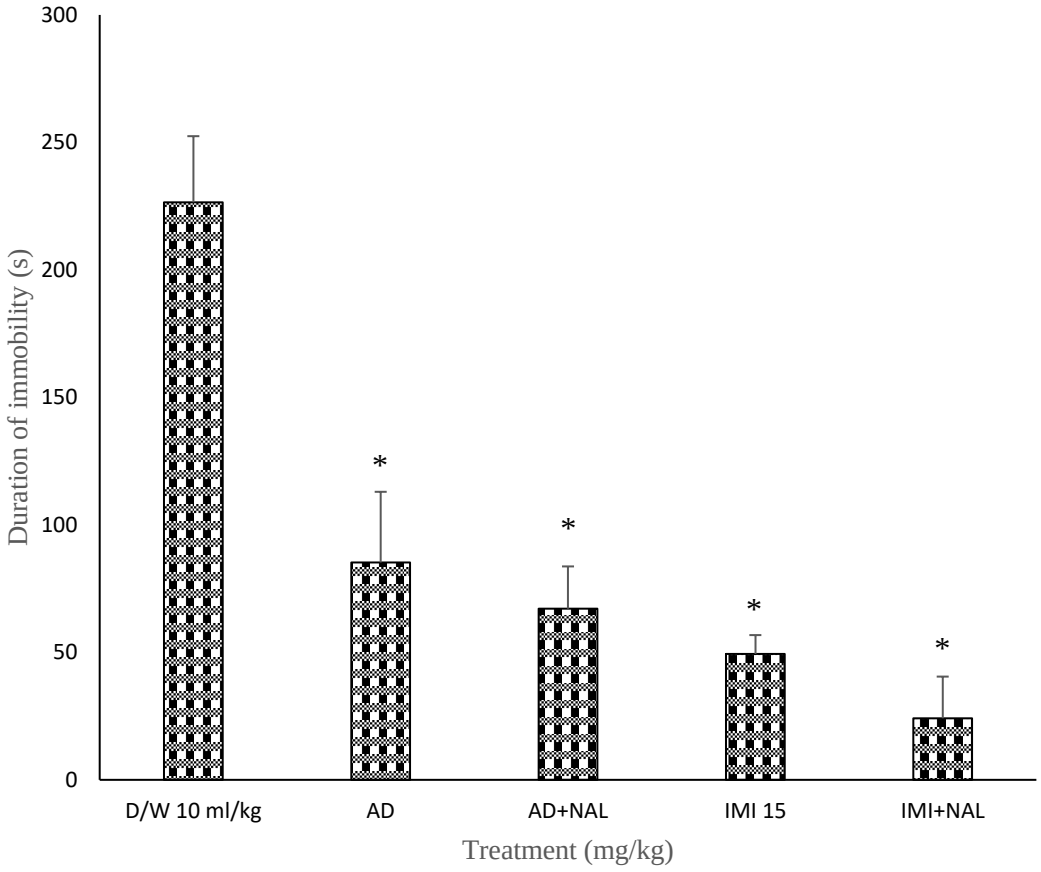


Figure 4.39: **Effect of Naloxone on Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata* in the Mouse Tail Suspension Test**

Each column represents the mean ± SEM, n= 8. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *= $p \leq 0.001$ significantly different from DW group; AD= *Adansonia digitata* (1, 000 mg/kg, *po*); IMI= Imipramine (15 mg/kg, *po*), DW= Distilled water (10 ml/kg, *po*), NAL= Naloxone (2 mg/kg, *ip*)

CHAPTER FIVE

5.0 DISCUSSION

The present study firstly established a collection of medicinal plants commonly used in the management of depression among Hausa tribe of Zaria Kaduna State, Northwestern Nigeria. Secondly, thin layer chromatographic profile of phytoconstituents present in the surveyed medicinal plants was established. Thirdly, the oral acute toxicity of the surveyed medicinal plants was determined. Fourthly, the studies validated the folkloric claim and showed the specificity of the medicinal plants as antidepressant with no sedative action at the doses tested. Fifthly, the most active medicinal plant was found to be *Adansonia digitata* which showed significant dose dependent antidepressant activity, with no effect on cognition. Furthermore, the effect of the methanol stem bark extract was determined on chronic unpredictable mild stress induced depression, where it reversed the weight loss, inhibited locomotor activity (OFT), impaired cognition (NORT) and anhedonia (SPT) which is a core symptom in depression. Lastly, the mechanisms of action of MEAD was also evaluated and the neurotransmitter, metabolic, neurotrophic, nitric oxide and neuroendocrine systems were targeted.

Plants are more easily recognized by their local names in every part of the world. These local names play a vital role in ethnobotanical study of a specific tribe or region (Tugume *et al*., 2016). Respondents interviewed gave local names of plants in recipes for treating depression. The local names mentioned were authenticated with their respective botanical names using standard texts. Although local names are not recommended directly for scientific accounts of plants as they lack uniformity and consistency (Singh, 2008), yet they may certainly be considered as an important tool for obtaining useful information on plants especially if the information is derived from local people (Umair *et al*., 2017).

Local names provide means of reference by local people in a particular area (Erinoso and Aworinde, 2012). Information gathered showed that increasing number of people are turning to herbal remedies for the management of depression as evident in the awareness of depressive illness by the practitioners. The ten medicinal plants species disclosed by the respondents covered different plant forms. Trees were found to be the most used plants followed by climber, then bulb and shrub. Plants are important in traditional treatment of various diseases including depression (Adekunle, 2008). The result of this survey showed that majority (90%) of the herb sellers/traditional medical practitioners (TMPs)/herbalists claimed no occurrence of side effects following patients' use of herbal preparations. Some of the plants revealed in the survey have been cited in the ethnobotanical survey of some African countries (Jinju, 1990; Ayodele, 2005; Adekunle, 2008; Ajayeioba and Ogbole, 2010; Soladoye *et al*., 2010; Idowu *et al*., 2010; Oni, 2010) for other disease conditions. Thus, scientific studies on these plants would provide insights into their potentials and understanding the pharmacological actions of the active compounds found in them (Ramana, 2008; Mahwasane *et al*., 2013). Most of the recipes were obtained from a single plant source, for example *Caralluma dalzielli*, while others were in combinations with other plants. Infusions and grindings were the most frequently used methods of preparation for the ethno-surveyed plants. Only two of the plants were prepared using more than one method because of the fact that they can be used either fresh or dried. However, researches showed that there are quantitative and qualitative variations in the chemical components of fresh and dry plant materials (Okoh *et al*., 2008; Fatemeh *et al*., 2006). Thus, dried plant materials might not be as effective as freshly obtained herbs but even pharmacological experiment in laboratory employs drying techniques of plant parts to prepare plant extracts. Different solvents were reported to be used in this study for herbal remedy preparation. This could be based on the belief that some solvents are more

efficient in eluting the phytoconstituents present in a particular plant than others and depending on the plant parts. Extraction procedures and phytochemistry had confirmed the efficacy of one solvent over another as solvent of extraction in relation to the pharmacological actions (Muthaura *et al*., 2007).

This study has provided additional information on the relevance of plants in the treatment of depression in our society. It is a step forward towards investigating the medicinal plants diversity in Nigerian flora as well as collection of informational descriptions for the plants as reported previously (Sulaiman *et al*., 2015). This collection of information if developed and merged with scientific knowledge will serve as a mechanism for accessing, benefit-sharing and documenting traditional knowledge for sustainable socioeconomic development and poverty alleviation in Nigeria as proposed by Olajide (2003). There is a consensus among the respondents of their high patronage in the management of depression, this could be an indication of their effectiveness and affordability (Alfred, 2013). In addition to this, previous findings on the antidepressant potential of *Tapinanthus dodoneifolius* corroborated the assertion of the traditional practicioners (Foyet *et al*., 2014) as well as the finding in this study. Other plants like *Adansonia digitata* have been scientifically validated for several pharmacological activities including nutritional, anti-inflammatory, antibacterial and analgesic among others (De Caluwe *et al*., 2010; Rahul *et al*., 2015). The plant *Ficus platyphylla* has been evaluated for other central nervous system and neurological disorders such as epilepsy and sedation (Chindo *et al*., 2014).

The plants ethno-surveyed were also screened for phytochemical constituents using thin layer chromatography. TLC is one of the most popular and simple chromatographic technique used for separation of compounds. It is one of the quickest and most convenient means of assaying a large number of samples. In the phytochemical evaluation of herbal

drugs, TLC is being employed extensively to enable rapid analysis of herbal extracts with minimum sample clean-up requirement as well as to provide qualitative and semi quantitative information of the resolved compounds. Biological or pharmacological actions of plant extracts are known to be due to the presence of specific phytochemical constituents (Amin *et al*., 2012). Phytoconstituents like carbohydrates, saponins, flavonoids, tannins, alkaloids and terpenes were found present in the surveyed medicinal plants. Preliminary phytochemical screening gives a brief idea about the qualitative nature of active phytochemical constituents present in plant extracts (Evans and Trease, 2008), which helps the future investigators regarding the selection of the particular extract for further investigation or isolating the active principle (Mishra *et al.,* 2010; Barkaoui *et al*., 2017). The information on the presence or absence and the type of phytochemical constituents especially the secondary metabolites are useful taxonomic markers in identifying a particular species and distinguishing it from a related species, thus helping in the delimitation of taxa (Jonathan and Tom, 2008; Du *et al*., 2014). Each plant family, genus, and species produces a characteristic mix of these chemicals, and they can sometimes be used as taxonomic characters in classifying plants (BE, 2015)**.** The plants ethnosurveyed were found to have similar chemotaxonomic profile. It has been reported that the saponins and alkaloids possesses potent neuroactivity when tested for central nervous system activity (Dubois *et al*., 1998). Alkaloids are the most important secondary metabolites in many plants that are held responsible for their antidepressant activity (Elizabetsky and Costa-Campos, 2006). It is therefore plausible to suggest that the antidepressant activity may be due to the presence of alkaloid and saponins among other phytochemical constituents found present in the surveyed medicinal plant extracts.

Evaluating the toxicity profile of plants extract is essential in drug discovery. This helps in standardization of doses to be prepared and administered (Fennell *et al*., 2004). The

oral LD_{50} of most of the plants tested were found to be above 5,000 mg/kg indicating their relative safety. However, methanol extracts of *Ficus platyphylla*, *Caralluma dalzielli*, and *Olax manni* had oral LD_{50} of greater than 2,000 mg/kg, indicating that these plants are practically non toxic based on the scale proposed by Lorke (1983). The neurotoxic potentials of the plants were further assessed using beam walking assay. The number of foot slips made has been proposed as a sensitive measure which determines benzodiazepine-induced motor coordination deficits. So also, is a good predictor of doses producing clinical sedation (Yaro *et al*., 2015). Failure of the methanol plant extracts tested to produce foot slips in mice indicate that the extracts are devoid of sedative properties.

The forced swimming and tail suspension tests are behavioural despair tests utilized to ascertain pathophysiological mechanism of depression as well as to evaluate antidepressant drugs (Hsu *et al*., 2012). Both tests detect all major classes of antidepressant drugs like tricyclics, selective serotonin reuptake inhibitors and monoamine oxidase inhibitors (Matsuzaki *et al*., 2013). Indicator behaviour observed in both tests is behavioural despair characterized by immobility (Adem *et al*., 2012). This immobility reflects behavioural despair similar to the case in human depression (Ngoupaye *et al*., 2013, Hsu *et al*., 2015). Thus, the decreased immobility time exhibited by methanol extracts of the plants indicated its antidepressant-like activity. In addition to the above, psychostimulants such as amphetamine were shown to produce false positivity in the TST and FST. This in contrast to antidepressants, which do not produce a marked motor stimulation (Du *et al*., 2014). In order to rule out this activity, the methanol extracts of the medicinal plants tested were subjected to OFT. The inability of all the methanol extracts to increase line crossing behavior suggest that they do not increase ambulatory

behaviour of the animals. Plausibly, the decreased immobility of animals treated with all the methanol extracts may not be attributed to any stimulant potentials.

Based on the above findings from the preliminary antidepressant studies, the methanol extract of *Adansonia digatata* (MEAD) appeared to be the most promising and was selected for further antidepressant studies. The methanol extract of *Adansonia digatata* displayed consistent and specific antidepressant activity. The FST is the most utilized pharmacological model for screening novel antidepressants in rodents following acute treatment (Cryan *et al*., 2002). The test is considered sensitive and relatively specific for all types of antidepressants like tricyclics, serotonin selective reuptake inhibitors and MAO inhibitors (Detke *et al*., 1995). Although all antidepressant drugs were observed to reduce immobility in the FST, two distinctive behavioural patterns were reported. It has been practically ascertained that swimming is more with serotonergic compounds like fluoxetine, while climbing is linked to compounds like tricyclic antidepressants with selective effects on noradrenergic transmission (Vazquez-Palacios *et al*., 2004). In this study, treatment with MEAD dose dependently decreased immobility which was accompanied by increased swimming activity. This activity pattern showed by the MEAD is similar to those shown by agents that act via the serotonergic pathways. However, FST has not been viewed as a consistent model for observing selective serotonin reuptake inhibitory action, but are generally reported as active in the TST with greater pharmacological activity as compared to FST (Cryan *et al*., 2005). The ability of the MEAD to reduce duration immobility in the TST has further ascertain the antidepressant activity and the utilization of serotonergic pathways. Unlike MEAD, imipramine only enhanced climbing but not swimming time which ascertained the activity for tricyclic antidepressants.

Rodents are likely to interact more with a novel object than with a familiar object (Felice *et al*., 2018). This likelihood has been used by behavioural scientists to study learning and memory, and one of the protocols utilized for such study is the object-recognition task (Berlyne, 1950). Novel object recognition task has been used to study mutation, aging deficits, early developmental problems, nootropic manipulations, teratological drug exposure and novelty seeking (Bevins and Besheer, 2006; Arnsten *et al*., 2012). The extract MEAD showed no change in recognition memory. This showed that the plant had no effect on memory and learning capabilities following acute administration.

The chronic unpredictable mild stress (CUMS) model is a valid model used to mimic people challenged with unpredictable stress every day. The model is widely used to induce depression in rodents and to screen for potential antidepressant agents. The model was considered successful following stressor applications for two consecutive weeks, after which fewer locomotor behaviours, increased immobility time, weight losses and decreased sucrose consumptions were observed. Moreover, an increase in the level of an important neuroendocrine hormone, Cortisol in HPA axis pathway and the resultant depletion of brain derived neurotrophic factor (BDNF), an important growth factor in hippocampal neurogenesis were observed. In this study, three weeks' treatment with methanol extract of *Adansonia digitata* (MEAD) reversed these behavioural effects. On the other hand, treatment with imipramine reversed the altered sucrose consumption but not the impaired locomotion. Mice subjected to CUMS showed decrease sucrose consumptions which was an indication of anhedonia (Almeida *et al*., 2015), one of the key symptoms of depressive disorders. It has been postulated that depressed individuals show poor responses to rewards. The depressed mice after treated with either imipramine or MEAD have greater preference for sucrose than the CUMS mice; this result is in

agreement with previous works where the altered sucrose consumption was reversed by antidepressant therapy (Liu *et al*., 2014; Qiu *et al*., 2014; Wang *et al*., 2015; Zhai *et al*., 2015).

In the tail suspension test (TST), the unstressed mice attempted to escape the challenge, this made their immobility shorter than that of the stressed 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 (Shen *et al*., 2016). In this study, these behavioural symptoms were simulated by CUMS and reversed by treatment with imipramine and MEAD extract. Moreover, TST has advantage of allowing researcher to screen and appraise the antidepressant effects of new drugs while ruling out the issue of false positive and false negative associated with other test such as forced swim test (Oberholzer *et al*., 2017).

In the open field test, stressed mice displayed fewer locomotor activities when compared with the unstressed mice, indicating a loss of interest in external stimuli. However, treatment with MEAD reversed the impaired locomotor activity of the mice to normal without further stimulant action. This result supported the fact that the antidepressant action of MEAD is specific at doses tested and is not associated with any stimulating locomotor activity. On the other hand, imipramine did not reverse the altered locomotor activity. This was so because chronic administration of imipramine at doses of 10 mg/kg and above has been reported to inhibit locomotor activities in rodents (Duda *et al*., 2016).

The loss of body weight in CUMS mice indicated the validity of the model in this study. So also, the increase in body weights following MEAD and imipramine treatment showed

the antidepressant effect of the agents on CUMS. These findings are in line with some studies, which showed that antidepressants can reverse lost body weights in CUMS (Hurley *et al*., 2013; Jiang *et al*., 2012; Li *et al*., 2017).

In this study, BDNF concentrations were reduced in the brain of mice exposed to CUMS which was upregulated following treatment with MEAD. Neurotrophic system was assessed because it is reported worthy of attention in recent years in addition to the monoamine hypothesis (Jun *et al*., 2016, Liu *et al*., 2018; Wang *et al*., 2018). Brain derived neurotrophic factor plays a critical role in hippocampal neurogenesis, neuronal synaptic plasticity, and neuronal survival and differentiation (Lu *et al*., 2014). Several researches reported BDNF signaling pathway to be involved in CUMS induced depression (Qiu *et al*., 2014, Wang *et al*., 2015). It is well established that when BDNF binds to the ligand tropomyosin receptor kinase B, a dimer is formed followed by cascade of events. The formed dimer induces the phosphorylation of ERK and the downstream phosphorylation of CREB (Enoch *et al*., 2017). Phosphorylated CREB enhances the transcription of some target genes, including BDNF, and promotes the protein expression of BDNF (Yang *et al*., 2016; Wenda *et al*., 2016). The BDNF signaling pathway has been shown to be inhibited in rats subjected to CUMS, resulting in a reduction in hippocampal BDNF, p-ERK 1/2, and p-CREB protein levels (Urszula *et al*., 2013) which set pace for depressive episodes. The chronic administration of antidepressants result in a reversal of these molecular changes in the hippocampus, prevents neuronal apoptosis and promotes synaptic plasticity (Aiping *et al*., 2016, Chandrasekhar *et al*., 2017), thus symptoms of depression was ameliorated as observed with MEAD treatment.

The occurrence of depressive symptoms is closely related to decrease of monoamine neurotransmitters, which is involved in HPA-axis hyperactivity (Zhao *et al*., 2018). Under long-term chronic stress and social stress, HPA-axis hyperactivity can be induced, which has been found to cause reduction in mono-amine neurotransmitters in the central and peripheral nervous systems as well as increase in serum levels of cortisol (Pereira Juruena *et al*., 2018). The resultant disruption in the HPA-axis is closely linked with depressive behaviour. Antidepressants relieve HPA-axis hyperactivity, improve levels of monoamine neurotransmitter and also decrease the levels of excess cortisol in circulation. In this study, MEAD significantly reversed HPA-axis hyperactivity by reducing serum cortisol levels. Furthermore, the decrease in cortisol levels showed that MEAD remarkably upregulated the level of monoamine neurotransmitters (5-HT and NE), thus further ascertaining the involvement of the serotonergic and adrenergic pathways observed and discussed below. Therefore, the antidepressant effect of MEAD on CUMS mice may involve inhibition of the HPA axis and upregulation of monoamine neurotransmitters levels. However, the reduced hyperactivity of the HPA axis is at lower doses of MEAD implying that its effectiveness involves certain threshold which when exceeded may be ineffective.

Changes in superoxide dismutase (SOD, a peripheral antioxidant defences) activity and malonaldehyde (MDA, a lipid peroxidation marker) level in major depression have been reported in some clinical studies (Khajehnasiri *et al*., 2013, Bajpai *et al*., 2014). In animal models of chronic induced-stress, enhanced oxidative stress mainly expressed as increase in the product of lipid peroxidation (MDA level) and the resultant decrease in SOD activity has been reported (Zhao *et al*., 2008, Luca *et al*., 2009, Duda *et al*., 2016). In this study, the untreated stressed mice exhibited decreased erythrocyte total SOD activity and increased plasma MDA level. However, MEAD decreased the MDA level and also

increased the SOD activity. Superoxide dismutase (SOD) is a key enzyme in the dismutation of superoxide radicals resulting from cellular oxidative metabolism, converting them into hydrogen peroxide, and, as a result, serves a key antioxidant role (Bilici *et al*., 2001). Thus, the oxidant and antioxidant valance system when distorted needs treatment for its homeostasis (Galecki *et al*., 2007). Short term administration of antidepressant has little effect on stress biomarkers, while chronic administration of selective serotonin reuptake inhibitors stabilizes oxidative stress in valance state but not tricyclic antidepressants (Duda *et al*., 2016) as observed with imipramine treated group in this study. This effect could be due to the fact that imipramine is associated with high risk of hepatotoxicity following its prolong use (Voican *et al*., 2014) and this was proposed to be related to oxidative system imbalances (Duda *et al*., 2016). The imbalances could probably be due to additive pro-oxidative effects of CUMS and imipramine resulting from the stress-induced activation of hypothalamic—pituitary—adrenal (HPA) axis and sympathetic—adrenal medullary (SAM) axis as well as imipramine-induced increase in peripheral level of serotonin and noradrenaline (Wasik *et al*., 2013; Antkiewicz-Michaluk *et al*., 2014).

Most of the antidepressants agents available are associated with one shortcoming or another. As part of the target for future promising and novel antidepressants discovery is the manipulations of nitric oxide pathway (Mutlu *et al*., 2012) to develop novel and promising antidepressants. An attempt was also made in this study to assess the involvement of nitric oxide pathway. Nitric oxide plays a regulatory role in behaviour, cognition, learning and memory (Rafalovich *et al*., 2015). It is synthesized in the brain from a precursor L-arginine via the activities of an enzyme nitric oxide synthase (NOS) following excitatory amino acids activation of NMDA receptors (Moncada *et al*., 1991).

There are two major targets in this pathway, the first is utilization of nitric oxide synthase substrate like L- arginine to either reverse the antidepressant-like effect or worsen depression. The second target is utilization of the nitric oxide synthase inhibitor such as 1-(2-trifluoromethylphenyl)-imidazole (TRIM) to either augment antidepressant-like activity or produce antidepressant like-effect (Ulak *et al*., 2008). Pretreatment with L-arginine did not reverse the antidepressant activity of MEAD in this study. On the other hand, pretreatment with NOS inhibitor L-NNA augmented the effect of antidepressant activity of MEAD. NOS activity has been reported to be involved in the mechanism of action of antidepressants such as the selective serotonin reuptake inhibitor (SSRI) (Ghasemi *et al*., 2008). Selective serotonin reuptake inhibitors were reported to inhibit in vitro NOS activity with a resultant decrease in plasma nitrite and nitrate levels in depression (Finkel *et al*., 1996; Attari *et al*., 2016). Additionally, NOS inhibitor was also reported to increase serotonin levels in hippocampus following systemic administration (Moret *et al*., 2009). This showed that antidepressant-like effects of NOS inhibitors may be associated with alterations in brain serotonin levels.

The involvement of dopamine D_2/D_3 receptors in the antidepressant effects of MEAD was also evaluated using sulpiride a specific antagonist of the D_2 receptor (Antonaj *et al*., 1994). Sulpiride is a derivative of benzylamide highly selective for central and peripheral dopamine D_2 and D_3 receptors (Jenner and Marsden, 1984). It has both typical and atypical antipsychotic properties (Jenner and Marsden, 1982) as well as prolactin stimulatory action (Seeman and Van, 1994). The ability of sulpiride to reverse the antidepressant activity of methanol stem bark extract of *A. digitata* suggests that its activity may be mediated specifically via the D_2/D_3 dopaminergic pathway. This finding is corroborated by the reversal of the antidepressant activity of imipramine by sulpiride.

Imipramine is known to act via dopamine D_2 receptors (David *et al*., 1991) and this was evidently observed in this study.

Metergoline reversed the antidepressant activity of the extract indicating the involvement of serotonergic pathways. Imipramine on the other hand rarely act via serotonergic pathway in antidepressant activities, thus metergoline had no effect on it activity. Metergoline is a potent antagonist of 5-HT_1 receptor antagonist. The 5-HT_1 receptor is one of the most studied out of the fourteen serotonergic receptors (Pucadyil *et al*., 2005) and useful with regards to antidepressant actions (Mutlu *et al*., 2012).

Cyproheptadine is a serotonin (5-HT_2 receptor) antagonist that causes sedation and reverse the antidepressant effect of the SSRI but not TCAs (Victor, 1995). More so, it is an antihistamine used for allergic rhinitis, conjunctivitis and certain skin reactions in addition to its benefits in serotonin syndrome therapy (Hargrove and Kimberley, 2009). Serotonergic (5-HT_2) receptor especially the 5-HT_{2A} and 5-HT_{2c} subtypes are involved in neurochemical changes elicited by antidepressants (Miaomiao *et al*., 2017). A clear correlation has also been established between 5-HT_2 receptor polymorphism and suicidal attempts in severely depressed patients (Du *et al*., 2000) which ascertained the involvement of 5-HT_2 receptors in depression. In this study, pretreatment with cyproheptadine reversed the antidepressant-like effect of MEAD. The involvement of 5-HT_2 receptor in the mechanisms of action of MEAD is an indication that it might be useful in severely depressed individual with suicidal ideation.

Additionally, pretreatment with prazosin and yohimbine resulted in the reversal of the antidepressant activity of MEAD. Unlike the extract, the antidepressant effect of imipramine was reversed by yohimbine but not prazosin. Prazosin and yohimbine are antagonist of α_1 and α_2 adrenergic receptors respectively (Ales and Karel, 2008). Yohimbine act to block dopamine and serotonin and this results in worsening of depression (Heninger *et al*., 1988). There is evidence of increase cortisol response to the α_2-antagonist yohimbine which in turn increase hypothalamo-pituitary- adrenal (HPA) axis (Price *et al*., 1986). Yohimbine is also reported to enhance dopamine turnover and suppresses serotonin turnover of brain striatum. Furthermore, it reduces firing of serotonergic neurones in raphe nuclei (Mark *et al*., 2000). The ability of yohimbine to reverse the antidepressant effect of methanol extract of *A. digitata* further affirms the involvement of dopaminergic and serotonergic pathways in eliciting the antidepressant action. The antidepressant activity of MEAD may also be due to the suppression of HPA-axis activity which is usually hyper activated in depression. Several researches have also proposed the ability of yohimbine to abolish antidepressant activity of imipramine (Husain *et al*., 2015) as clearly confirmed in this study.

Prazosin is an alpha-adrenergic blocker used as antihypertensive, in heart failure and Raynaud's syndrome. It is also used in prostate hyperplasia and in scorpion stings with depression as one of its commonest side effect (Ben, 2013). Many clinical researches reported alterations in brain alpha-1 noradrenergic neurotransmission to be associated with some of the symptoms observed in depression and these alterations were linked to increased corticosteroid secretions. In this study, prior administration of prazosin resulted in the reversal of the antidepressant activity of MEAD. This showed the involvement of α_1 adrenergic receptors in antidepressant action of MEAD probably via increase in α_1

noradrenergic neurotransmission or via decrease corticosteroid secretions. On the other hand, prazosin does not alter the effect of imipramine as previously reported (Zarrindast *et al*., 2003).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Depression was observed to be known by traditional medical practitioners in Zaria, Nigeria where medicinal plants are used locally in the management of depression. A collection of medicinal plants used in the management of depressive illnesses by the Hausa tribe of Zaria was established.

Thin layer chromatographic finger prints as phytochemical profiles of these medicinal plants were established to possess constituents like saponins, tannins, steroids, flavonoids, alkaloids and even anthraquinones in some of the plants.

Oral acute toxicity studies of the methanol extracts of the medicinal plants investigated were relatively safe.

Pharmacological studies provided some scientific rationale of the medicinal plants use in the management of depression and their specificity as antidepressant with no sedative effect was established at the doses tested.

Adansonia digitata was found at to be most promising with specificity as antidepressant and was further subjected to test on cognitive behaviour and chronic unpredictable mild stress (CUMS)-induced depression. The methanol stem bark extract of *A. digitata* showed no significant effect on cognition acutely, however, reversed the impaired cognition, weight loss and anhedonia induced by CUMS-induced depression following three weeks' treatment.

The study established the possible mechanism of actions of MEAD, which include the involvement of monoaminergic, metabolic, neurotrophic, nitric oxide and neuroendocrine systems.

6.2 Conclusion

The study established a collection of medicinal plants used in the management of depression by traditional medical practitioners in Zaria and their use in the management of depression was validated. Phytochemical constituents found in the chromatographic finger print may be responsible for the observed activity. The acute toxicity studies revealed the relative safety of the plants. The plant *Adansonia digitata* was found to be very promising which reversed the chronic mild stress induced depression and elicited antidepressant activity via the monoaminergic, neurotrophic, metabolic, nitric oxide and neuroendocrine systems.

6.3 Recommendations

1. Further antidepressant studies should be conducted on the other medicinal plants.
2. The activity of *Adansonia digitata* on inflammatory pathways and down stream mediators of depression as the possible mechanism of action should also be considered.
3. Bioassay guided fractionation should be conducted to isolate and characterize the bioactive constituents responsible for the antidepressant activity of *Adansonia digitata*.

6.4 Contribution to Knowledge

1. The study established a collection of medicinal plants used in the management of depression by traditional medical practitioners of Zaria, Nigeria.
2. The study provided some scientific rationale for the folkloric use of the surveyed medicinal plants in the management of depression.

3. The study showed *Adansonia digitata* as the most promising medicinal plant, with specific antidepressant action on chronic unpredictable mild stress-induced depression and no effect on cognition.
4. The study established the possible mechanisms of action of methanol stem bark extract of *Adansonia digitata* which may involve neurotransmitter (dopaminergic, serotonergic, adrenergic, opioidergic and muscarinic), neurotrophic (brain derived neurotrophic factor), metabolic (oxidant and antioxidant), nitric oxide and neuroendocrine (cortisol) systems.

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APPENDICES

Appendix I: Questionnaire Administered During the Ethnobotanical Survey of Medicinal Plants used in the Management of Depression among Hausa tribes in Northwestern Nigeria

A QUESTIONNAIRE ON THE ETHNOBOTANICAL SURVEY OF PLANTS USED IN THE MANAGEMENT OF DEPRESSION IN NORTHWESTERN NIGERIA

Please answer all questions as honest as possible. It's strictly voluntary and all information is confidential. Thank you for your time and cooperation.

SECTION 1; Demographic information

1. Age

   10-20 [ ] 21-30 [ ] 31-40 [ ] 41 and above [ ]

2. Sex

   Female [ ] Male [ ]

3. Religion

   Islam [ ] Christian [ ] Traditional [ ] others specify [ ] ……

4. Nationality

   Nigerian [ ] others specify [ ] ……..

5. Practice specification

   Herbalist [ ] Herb seller [ ] Traditional medical practitioner [ ]

6. Duration of practice

   0-5 yrs [ ] 6-10 yrs [ ] 11-15 yrs [ ] 15-20 yrs [ ] 21 yrs and above [ ]

7. Educational background

   Primary [ ] Secondary [ ] Tertiary [ ] None [ ]

   SECTION 2; Professional experience of the treatment of depression

8. Type of depression treated

9. Frequency of treatment

10. Use of herbal therapy alone or otherwise

11. Duration of treatment

12. Accompanied side effects

13. Accompanied verbal instructions

14. Plant part(s) use

15. Availability of plant or plant part(s) and knowledge of treatment

SECTION 3; Plants use in the treatment of depression

16. Plants and recipes used in the treatment of depression

17. Herbal preparation used in the treatment of depression

18. Arrangement of plant part(s) ingredient

19. Traditional solvent of choice
Water [ ] Alcohol [ ] Honey [ ] Oil [ ]

20. Traditional extraction method or method of preparation
Maceration [ ] Percolation [ ] Boiling [ ] Shaking [ ] Digestion [ ]

21. Method of administration
Oral [ ] Topical [ ] Inhalation [ ] Irrigation [ ]

Appendix II: Ethical Clearance Certificate from Ahmadu Bello University Committee on Animal Care and Use, Ahmadu Bello University, Zaria.

Appendix III: List of Publications in Peer Reviewed Journals

i. **Shehu A.**, Magaji M.G., Yau, J. and Abubakar, A. (2017). Ethnobotanical survey of medicinal plants commonly used in the management of depression by Hausa tribes of Kaduna State, Nigeria. ***Journal of Medicinal Plant Research***, 11(36): 562-567.

ii. **Shehu Aishatu**, Ngbara Ummi, Magaji Muhammad Garba, Yau Jamilu and Ahmed Abubakar (2018). Antidepressant activity of methanol stem bark extract of *Ficus platyphylla* Del (Moraceae) in mice. ***Journal of Herbal Drugs***, 8(4): 227-234.

iii. **Shehu, A.,** Magaji, M.G., Yau, J., Mahmud, B. and Ahmed, A. (2018). Antidepressant Effect of Methanol Stem Bark Extract of *Adansonia digitata L. (Malvaceae)* in Mice. ***Tropical Journal of Natural Product Research***, 2(2): 87-91.

iv. **Shehu, A.,** Magaji, M.G., Yau, J. and Ahmed, A. (2017). Antidepressant-like Effect of *Tapinanthus globiferus* Vantiegh (A. Rich) in Mice. ***Yusuf Maitama University Journal of Pure and Applied Science***, 3(3): 1-6.

Appendix IV: Effect of Methanol Extract of the Medicinal Plants Used in the Management of Depression on Immobility of Mice in the Tail Suspension Test

Treatment	Dose (mg/kg, *po*)	Duration of Immobility (s)
Distilled water	10 ml/kg	226.50 ± 25.94
Imipramine	15	56.25 ± 22.79***
Adansonia digitata	250	129.38 ± 11.86**
	500	125.88 ± 6.30**
	1000	85.25 ± 27.71***
Combretum micranthum	250	121.25 ± 10.69**
	500	121.25 ± 10.69**
	1000	115.88 ± 15.30**
Caralluma dalzielli	125	129.50 ± 4.45*
	250	105.00 ± 20.61**
	500	101.00 ± 20.77***
Ficus platyphylla	125	133.13 ± 27.11*
	250	110.88 ± 3.94**
	500	109.25 ± 8.08**
Olax manni	125	128.75 ± 11.47**
	250	104.63 ± 19.72***
	500	115.50 ± 8.45**
Pancrantum micranthum	250	122.38 ± 27.05*
	500	100.38 ± 18.83**
	1000	99.63 ± 17.23**
Senna occidentalis	250	105.75 ± 19.43**
	500	127.25 ± 18.81*
	1000	84.25 ± 17.65***
Tapinanthus dodeinofolius	250	122.75 ± 24.92**
	500	140.25 ± 88.0*
	1000	140.25 ± 19.02***
Tapinanthus globiferus	250	112.13 ± 25.52**
	500	194.63 ± 16.06
	1000	74.13 ± 14.10**

Data presented as mean±SEM. Oneway ANOVA followed by Bonferoni post hoc test, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$ significant difference as compared to distilled water group.

Appendix V: Effect of Methanol Extract of the Medicinal Plants Used in the Management of Depression on Locomotor Activity of Mice in the Open Field Test

Treatment	Dose (mg/kg, *po*)	Number of Line Crossing
Distilled water	10 ml/kg	108.75 ± 8.39
Diazepam	10	60.38 ± 22.79**
Adansonia digitata	250	91.75 ± 14.01**
	500	130.75 ± 13.93
	1000	90.88 ± 14.1
Combretum micranthum	250	101.13 ± 6.55
	500	101.13 ± 6.55
	1000	111.88 ± 7.38
Caralluma dalzielli	125	66.88 ± 8.42**
	250	77.75 ± 12.85
	500	84.00 ± 7.07
Ficus platyphylla	125	91.0 ± 11.22
	250	92.88 ± 7.91
	500	86.25 ± 10.24
Olax manni	125	71.75 ± 10.17*
	250	77.13 ± 14.13
	500	151.88 ± 1.86
Pancrantum micranthum	250	111.00 ± 17.82
	500	109.5 ± 17.61
	1000	99.00 ± 12.06
Senna occidentalis	250	124.63 ± 5.41
	500	55.88 ± 18.74**
	1000	78.00 ± 12.79*
Tapinanthus dodeinofolius	250	74.63 ± 10.92
	500	119.75 ± 9.61
	1000	66.50 ± 11.31*
Tapinanthus globiferus	250	61.25 ± 6.48*
	500	95.88 ± 14.17
	1000	107.25 ± 19.60

Data presented as mean±SEM. Oneway ANOVA followed by Bonferoni post hoc test, *$p \leq 0.05$, **$p \leq 0.01$, significant difference as compared to distilled water group.

Appendix VI: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Immobility of Mice in the Forced Swim Test

Treatment	Dose (mg/kg, *po*)	Duration of Immobility
Distilled water	10 ml/kg	241.50 ± 7.10
Imipramine	15	77.38 ± 26.10*
Adansonia digitata	250	34.50 ± 10.85*
	500	5.63 ± 13.93*
	1000	5.00 ± 1.98

Data presented as mean±SEM. Oneway ANOVA followed by Bonferoni post hoc test, *$p \leq 0.001$ significant difference as compared to distilled water group.

Appendix VII: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Swimming and Climbing Behaviour of Mice in the Forced Swim Test

Treatment	Dose (mg/kg, *po*)	Swimming (s)	Climbing (s)
Distilled water	10 ml/kg	56.64 ± 6.42	2.00 ± 1.00
Imipramine	15	110.75 ± 26.67	64.88 ± 17.07*
Adansonia digitata	250	265.50 ± 10.85*	32.88 ± 2.92
	500	269.63 ± 11.80*	25.38 ± 5.68
	1000	292.00 ± 6.02*	23.13 ± 3.32

Data presented as mean±SEM. Oneway ANOVA followed by Bonferoni post hoc test, *$p \leq 0.01$ significant difference as compared to distilled water group.

Appendix VIII: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Exploration Time of Novel and Familiar Objects in the Novel Object Recognition Task in Mice

		Exploration Time of Objects (s)	
Treatment	**Dose (mg/kg, *po*)**	**Familiar**	**Novel**
Distilled water	10 ml/kg	45.75 ± 2.24	54.25 ± 2.24
Imipramine	15	48.38 ± 7.29	39.13 ± 6.05*
Adansonia digitata	250	38.63 ± 4.88	61.38 ± 4.88
	500	41.86 ± 10.06	58.14 ± 10.06
	1000	40.71 ± 5.65	59.74 ± 2.89

Data presented as mean±SEM. Oneway ANOVA followed by Bonferoni post hoc test, *$p \leq 0.05$ significant difference as compared to distilled water group.

Appendix IX: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Discrimination Index of Mice in the Novel Object Recognition Task

Treatment	Dose (mg/kg, *po*)	Discrimination Index (%)
Distilled water	10 ml/kg	7.5 ± 2.41
Imipramine	15	14.00 ± 4.86
Adansonia digitata	250	31.3 ± 5.45
	500	43.57 ± 11.49*
	1000	19.71 ± 10.97

Data presented as mean±SEM. Oneway ANOVA followed by Bonferoni post hoc test, *$p \leq 0.05$ significant difference as compared to distilled water group.

Appendix X: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Weights of Mice following the Chronic Unpredictable Mild Stress

Treatment	Mean Weights					
(mg/kg)	0 week	1 week	2 weeks	3 weeks	4 weeks	5 weeks
NOCUMS	18.36±0.79	21.09±0.66	22.55±0.56	23.96±0.58	25.55±0.54	27.0±0.50
CUMS	19.50±0.93	18.58±0.78	17.38±0.66$^{\#}$	16.98±0.68$^{\#\#}$	15.10±0.63$^{\#\#}$	14.34±0.59$^{\#\#}$
CUMS+IMI 15	19.38±0.93	17.98±0.78	17.04±0.66$^{\#}$	17.74±0.68$^{\#}$*	17.75±0.63$^{\#}$*	19.61±0.59$^{\#}$*
CUMS+AD250	18.44±0.88	17.59±0.73	16.26±0.62$^{\#}$	16.86±0.64$^{\#\#}$	17.40±0.60$^{\#}$*	18.22±0.56$^{\#}$*
CUMS+AD500	19.11±0.88	17.76±0.73	16.72±0.62$^{\#}$	17.38±0.64$^{\#\#}$	18.02±0.6$^{\#}$*	19.28±0.56$^{\#}$*
CUMS+AD1,000	19.33±0.88	18.27±0.73	16.93±0.62$^{\#}$	17.56±0.64$^{\#\#}$	18.19±0.60$^{\#}$*	19.33±0.56$^{\#}$*

The body weights following CUMS in mice were recorded six times, once every week. Data were analysed using Repeated measures ANOVA followed by Bonferoni post hoc test, presented as mean ± SEM (n = 8 - 12). $^{\#}p \leq 0.01$ & $^{\#\#}p \leq 0.001$ significant difference as compared to NOCUMS group; $^{*}p \leq 0.05$ significant difference as compared to CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*)

Appendix XI: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Sucrose Consumption of Mice following Chronic Unpredictable Mild Stress

Treatment (mg/kg)	Sucrose Consumption (mls)		
	0 week	2 weeks	5 weeks
NOCUMS	11.99 ± 1.42	14.75 ± 1.13	15.68 ± 1.37
CUMS	11.95 ± 1.30	7.09 ± 0.56$^{\#}$	5.27 ± 0.64#
CUMS+IMI 15	11.79 ± 1.34	6.86 ± 0.60$^{\#}$	12.15 ± 1.55*
CUMS+AD250	11.48 ± 1.28	8.17 ± 0.48$^{\#}$	12.07 ± 1.67*
CUMS+AD500	11.78 ± 0.98	7.13 ± 0.95$^{\#}$	12.78 ± 1.71*
CUMS+AD1,000	12.78 ± 1.49	6.5 ± 0.65$^{\#}$	12.17 ± 1.70*

Results were represented as mean ± SEM (n = 8-12). Data were analysed using one-way ANOVA followed by Bonferroni post hoc test, $^{\#}p \leq 0.05$, $^{\#\#}p \leq 0.01$ significant difference as compared to NOCUMS group; $^{*}p \leq 0.001$ significant difference as compared to CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*)

Appendix XII: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Line Crossing Behaviour of Mice following Chronic Unpredictable Mild Stress

Treatment (mg/kg)	Line Crossing		
	0 week	2 weeks	5 weeks
NOCUMS	95.50 ± 7.68	134.30 ± 7.57	107.90 ± 10.65
CUMS	98.25 ± 9.81	80.90 ± 5.18$^{\#}$	37.40 ± 9.20$^{\#}$
CUMS+IMI 15	94.08 ± 6.04	93.00 ± 5.36$^{\#}$	65.95 ± 5.51$^{\#}$
CUMS+AD250	96.17 ± 6.10	90.90 ± 5.36$^{\#}$	89.40 ± 9.57*
CUMS+AD500	95.50 ± 5.24	89.10 ± 3.41$^{\#}$	105.60 ± 5.51**
CUMS+AD1,000	98.25 ± 9.81	92.50 ± 4.62$^{\#}$	102.70 ± 5.74**

Data were analysed using one-way ANOVA followed by Bonferoni post hoc test, expressed as mean ± SEM (n = 8-12). $^{\#}p<0.05$ significant difference as compared to NOCUMS group, $*p\leq 0.05$, $**p\leq 0.01$ significant difference as compared with CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*)

Appendix XIII: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on Immobility of Mice following Chronic Unpredictable Mild Stress

Treatment (mg/kg)	Duration of Immobility		
	0 week	2 weeks	5 weeks
NOCUMS	125.83 ± 8.14	123.13 ± 10.17	138.13 ± 15.27
CUMS	141.50 ± 12.30	163.83 ± 6.45$^{\#}$	194.33 ± 12.21$^{\#}$
CUMS+IMI 15	123.75 ± 9.35	159.50 ± 9.15$^{\#}$	121.63 ± 11.95*
CUMS+AD250	127.50± 11.28	153.60 ± 8.85$^{\#}$	114.00 ± 18.01*
CUMS+AD500	119.00 ± 16.41	166.33 ± 7.31$^{\#}$	109.89 ± 12.24**
CUMS+AD1,000	116.90 ± 16.41	153.00 ± 10.23$^{\#}$	93.63 ± 13.42**

All results were expressed as mean ± SEM (n = 8-12). Data were analysed using one-way ANOVA followed by Bonferoni post hoc test, #p < 0.05 significant difference as compared to NOCUMS group; *$p\leq$ 0.01, **$p\leq$ 0.001 significant difference as compared to CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1, 000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*)

Appendix XIV: Effect of Methanol Stem Bark Extract of *Adansonia digitata* on BDNF Concentration, Cortisol levels, MDA Concentration and SOD Activity following Chronic Unpredictable Mild Stress

Treatment (mg/kg)	Cortisol Conc (ng/mls)	BDNF Conc (Pg/ml)	MDA Conc (nMol/ml)	SOD Activity (µ/gHb)
NOCUMS	52.98 ± 11.55	1006.93 ± 134.46	30.98 ± 3.62	34.41 ± 2.54
CUMS	189.83 ± 33.32$^{\#\#}$	347.84 ± 100.47$^{\#\#}$	51.03 ± 3.48$^{\#}$	17.41 ± 1.38$^{\#}$
CUMS+IMI 15	23.44 ± 6.72*	826.11 ± 53.09***	44.71 ± 1.89$^{\#}$	21.43 ± 1.02$^{\#}$
CUMS+AD250	83.97 ± 27.57	807.62 ± 30.02*	34.01 ± 4.86*	32.94 ± 5.21*
CUMS+AD500	85.22 ± 34.06	832.69 ± 54.17**	33.60 ± 4.31*	33.89 ± 3.49**
CUMS+AD1,000	91.25 ± 24.81	1117.51 ± 165.08**	32.85 ± 3.50*	37.50 ± 1.99***

All results were expressed as mean ± SEM (n = 8). $^{\#\#}p\leq 0.05$ $^{\#}p\leq 0.01$ significant difference as compared to NOCUMS group; $*p\leq 0.05$, $**p\leq 0.01$, $***p\leq 0.001$ significant difference as compared to CUMS group. CUMS= Chronic unpredictable mild stress + distilled water (10 ml/kg, *po*), NOCUMS= No chronic unpredictable mild stress, AD= *Adansonia digitata* (250, 500 & 1,000 mg/kg, *po*), IMI= Imipramine (15 mg/kg, *po*), Conc= Concentration, BDNF= Brain Derived Neurotrophic Factor, MDA= Malondialdehyde, SOD= Superoxide dismutase

Appendix XV: **Involvement of Monoaminergic and Nitritergic System in the Antidepressant Activity of Methanol Stem Bark Extract of *Adansonia digitata***

Systems	**Treatment (mg/kg, route)**	**Duration Immobility (s)**
Distilled water	10 ml/kg, *po*	226.50 ± 25.94
Imipramine	15, *po*	49.38 ±16.57**
AD	1,000, *po*	85.25 ± 27.71**
Nitric Oxide Pathway	AD+L-Arginine (50, *ip*)	99.50 ± 11.74**
	IMI+L-Arginine (50, *ip*)	70.13 ± 12.5**
	AD+L-NNA (50, *ip*)	72.88 ± 28.17*
	IMI+L-NNA (50, *ip*)	16.75 ± 6.22**
Dopaminergic System	AD+Sulpiride (50, *ip*)	191.25 ± 20.2[a]
	IMI+Sulpiride (50, *ip*)	181.38 ± 31.59[b]
Noradrenergic System	AD+Prazosin (1, *ip*)	161.63 ± 7.45
	IMI+Prazosin (1, *ip*)	93.88 ± 14.65*
	AD+Yohimbine (1, *ip*)	151.25 ± 9.24
	IMI+Yohimbine (1, *ip*)	140.50 ± 18.18[a]
Serotonergic System	AD+Metergoline (1, *ip*)	140.25 ± 37.55
	IMI+Metergoline (1, *ip*)	48.50 ± 18.82**
	AD+Cyproheptadine (3, *ip*)	140.50 ± 13.16
	IMI+Cyproheptadine (3, *ip*)	77.50 ± 22.20**
Cholinergic System	AD+Atropine (1, *ip*)	39.25 ± 16.01*[a]
	IMI+Atropine (1, *ip*)	39.00 ± 16.93**
Opioidergic System	AD+Naloxone (2, *ip*)	67.13 ± 7.39*
	IMI+Naloxone (2, *ip*)	24.13 ± 16.36**

Each column represents the mean ± SEM of 8 animals. Data was analysed using One-way ANOVA followed by Bonferroni post hoc test, *$p \leq 0.05$, **$p \leq 0.001$ significantly different from DW treated group; a & b= $p \leq 0.01$ significantly different from AD and IMI treated groups respectively; AD= *Adansonia digitata* (1,000 mg/kg, *po*)