ANTIDEPRESSANT STUDY ON METHANOL LEAF EXTRACT OF ZIZIPHUS MAURITIANA LAM IN SWISS ALBINO MICE

BY

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DECLARATION

I declare that the work in this dissertation entitled, “Antidepressant Studies on Methanol Leaf Extract of *Ziziphus Mauritiana* in SwissAlbino Mice” has been carried out by me in the Department of Human Physiology. The information derived from literature has been duly acknowledged in the text and a list of references provided. No part of this dissertation was previously presented for another degree at this or any other institution.

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Signature Date

CERTIFICATION

This dissertation titled, “ANTIDEPRESSANT STUDIES ON METHANOL LEAF EXTRACT OFZIZIPHUS MAURITIANA LAM IN SWISS ALBINO MICE” by Maimunatu Muhammad KABIR meet the regulations governing the award of the degree of M.Sc. Human Physiology of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work has been dedicated to my late brother, Nauwasi Muhammad Kabir, myhusband whose love, moral and financial support saw me throughout the course of this work, my lovely children Taufiq, AbdulRafiq, AbdulFattah, Sulaiman jibril,Zainab Lawal and my parents whose prayer and support saw me throughout my entire life. May Allah (SAW) continue to blessed, guided and protect us. Ameen.

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ABSTRACT

The leaves of*Ziziphus mauritiana* have been reported to be used in the management of depressive illnesses in traditional medicine. The antidepressant activity of the methanol leaf extract of the plant was evaluated using tail suspension test (TST) and forced swim test (FST) at the doses of 25, 50, 100, and 200 mg/kg.Imipramine (20mg/kg) was used as standard antidepressant drug.The effect of the extract on recognition memory, motor coordination, and exploration was evaluated using novel object recognition test (NORT), beam walking assay test (BWAT) and open field test (OFT) respectively. In order to establish the involvement of inflammatory system in the antidepressant activity of the extract using *Bacillus Calmette-Guérin*(BCG) induced depressive like behaviour modelthrough chronic activation of the immune system in the chronic study, mice were inoculated with BCG (0.2 mg/kg, *i.p*) and later treated with the graded doses of the extract for 21 days. Body weight was measured 4 days before the inoculation and on daily based after, while the body temperature was recorded hourly. Exploration, immobility time and sucrose consumption were all assessed using open field test, tail suspension test and sucrose preference test respectively.Data wererepresented as± SEM and analyzed using one-way (treatment) or two-way (treatment × time) analysis of variance (ANOVA), followed by the Bonferroni *post hoc* test where$P < 0.05$ was considered significant .The results showed a statistical significant ($p < 0.001$) decrease in duration of immobility, significant ($p < 0.001$) increase in sucrose consumption,significant ($p < 0.001$) decrease in body temperature,significant ($p < 0.001$) increase in body weight in the extract treated groups when compared with the BCG + distilled water. The LD50 of the extract was >5000 mg/kg and phytochemical screening revealed the presence of flavonoids, alkaloids, tannins, cardiac glycoside, and saponins. It was concluded that the methanol leaf extract of *Ziziphus mauritiana*

possesses antidepressant activity, it also attenuated anhedonia, reversed body weight loss and produced antipyretic effect induced by BCG induced depressive like behaviour.

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LIST OF ABBREVIATIONS

MDD	Major Depressive Disorder
DSM-IV-TR	*Diagnostic and Statistical Manual of Mental Disorders*
WHO	World Health Organization
USA	American Psychiatric Association
ZZM	*Ziziphus mauritiana* plant
JuA	Jujuboside A
TST	Tail Suspension Test,
OFT	Open Field Test,
FST	Forced Swim Test,
NORT	Novel Object Recognition Test
SPT	Sucrose Preference Test
SAD	Seasonal Affective Disorder
HIV	Human Immunodeficiency Virus
PFC	Prefrontal cortex
GABA	Gamma Amino butyric Acid
BCG	*Bacillus* Calmette-Guérin
CNS	Central nervous system

LPS	Lipopolysaccharide
ANOVA	Analysis of variance
DPP IV	Dipeptidyl-peptidase IV)
HPA axis	Hypothalamic–pituitary adrenal
IDO	Indoleamine-2,3-dioxygenase
5-HT	Serotonin
IRS	Inflammatory Response System
BDNF	Brain-Derived Neurotropic Factor
LTP	Long term potentiation
SSRIs	Selective serotonin reuptake inhibitors
NE	Norepinephrine (NE)
DA	Dopamine
TCAs	Tricyclic Antidepressants
MAOIs	Monoamine oxidase inhibitors (MAOIs)
NDRIs	Norepinephrine dopamine reuptake inhibitor
SNRIs	Serotonin norepinephrine reuptake inhibitor
NRI	Norepinephrine reuptake inhibitor
NA	Nucleus Accumbens
BF	Basal forebrain

SC	Spinal cord
NT	Neurotransmitter
DW	Distilled water
CMS	Chronic mild stress

CHAPTER ONE

1.1 Introduction

Depression is a neuropsychiatric disorder affecting a huge percentage of the active population especially in developed countries (Neto *etal.*,2011). Itis an affective state of negative mood and low arousal that presents with depressed mood, loss of interest or pleasure, decreased energy, feelings of guilt or low self-worth, disturbed sleep or appetite, and poor concentration. Moreover, it often comes with symptoms of anxiety. These problems can become chronic or recurrent and lead to substantial impairments in an individual ability to take care of his or her everyday responsibilities. At its worst, depression can lead to suicide(WHO 2012). According to the current text revision version of the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-V), depression constitutes an episode of major depressive disorder (MDD) if it is denoted by at least five of nine symptoms: depressed mood, anhedonia, significant weight loss (or gain), or a decrease (or increase) in appetite, insomnia (or hypersomnia), psychomotor retardation (or agitation), fatigue or loss of energy, feelings of worthlessness or guilt, diminish ability to concentrate; and recurrent thoughts of death (not just fear of dying), or suicidal thoughts or actions (American Psychiatric Association, 2013). Additionally, the episode must last for at least 2 weeks, and it must cause substantial distress or impairment in an important domain of functioning. Finally, the episode must not be better accounted for by bereavement (which assumes that the episode is not disordered if it causes significant impairment for up to 2- months), and must not be caused by a substance or a medical condition (Carlson, 2013).

Major depression or Major depressive disorder (MDD) is a very common mental disorder, mainly characterized by persistent anhedonia (inability to experience pleasure), apathy or lack of

motivation, anxiety, and sadness (Belzung, 2014).It is a complex psychiatric disorder characterized by affective, cognitive, and physiological impairments that lead to maladaptive behaviour. Millions of people are affected by depression each year. According to the World Health Organization (2008), MDD was ranked as the third leading cause of disability worldwide, and is predicted to worsen as it is expected to become the number one cause of the global burden of disease by 2030 (World Health Organization 2012).Approximately 6.6% of adults, or about 13 million of the adult US population, are estimated to have had episodes that meet current criteria for Major Depressive Disorder (MDD) within a year's time (Kessler *et al.*, 2007). MDD occurs with a high prevalence. The 12-month prevalence in the United States is approximately 7%, with females experiencing 1.5–3-fold higher rates than males (USA, American Psychological Association, 2013).

Most episodes that meet diagnostic criteria for MDD are associated with stressors (e.g., bereavement, marital difficulties, interpersonal conflict, financial difficulties, and health issues). Only 11.9% of episodes appeared to be endogenous (depression in the absence of an environmental trigger). However, even many of the apparently endogenous episodes could have reflected stressors of a sensitive nature (Leff*et al.*, 1970).

1.2 *Ziziphus mauritiana*Plant

Ziziphus mauritiana or Jujubes is an important tropical fruit tree with a wide profile of putative therapeutic applications .It is a species of the genus *Ziziphus* tourn that belongs to the family Rhamnaceae. The name *Ziziphus is* related to an Arabic word and ancient Greeks used the word ziziphon for the jujube, Indian jujube, or Indian plum, Chinese apple, or ber in English,

(Morton,2009), it is calledMagarya in Hausa.*Ziziphus mauritana* fruits have highly useful phytochemical contents that is useful for human health (Parmar *et al.,* 2012).

Taxonomicalclassification of the plant

Kingdom:Platae

Division:Magnoliophyta

Class:Magnoliopsida

Order:Rosales

Family:Rhamnaceae

Genus:Ziziphus

Species: Jujuba(Preeti *etal*., 2014)

This plant has various uses in traditional medicine for instance the fruits are applied on cuts and ulcers, and are employed in pulmonary ailments and fevers, rheumatism, and are mixed with salt and chili pepper, and given in indigestion (Najafi *et al.,* 2013). An infusion of the flower serves as an eye lotion and is also useful in curing eye diseases (Beg *etal*., 2016).

The leaves of *Ziziphus mauritiana* are also found to have antipyretic effect and are helpful in reducing obesity. The fruit improves digestion,removes biliousness, possess aphrodisiac and laxative effect and has been used widely for their good response in the treatment of tuberculosis, blood diseases and burning sensations. It is also explored as an antidote to aconite poisoning and abdominal pain in pregnancy(Beg *etal.,*2016).The traditional workers of Chhattisgarh, India usethe fruit to treat common fevers and use the seeds with bar sprouts *(Ficus benghalensis)* and sugar for vomiting (Janick and Paul, 2009). Roots are used with cow's milk to treat dysentery until the patient is cured. The traditional healers use the fresh leaves with cumin to treat urinary infections (Han and Park, 1986).The seeds and leaves of many *Ziziphus* species have been found

to possessanxiolytic and hypnotic-sedative effects. Both seeds and leaves are known to depress activity of the central nervous system which reduces anxiety and induces sleep (Peng *et al.*,2000), and are used in treatment of convulsion, epilepsy, and insomnia (Samina*et al.*, 2013). Anxiolytic effects in mice of a polyherbal substance containing seed extract of *Z. jujube has* beenreported *(*Liu *et al.*, 2003).



Plate 1 ***Ziziphus mauritiana*** **Plant (Snapped by the Candidate, 2017)**

Chemically, it contains carbohydrate, starch, amino acidproteins, mucilage, minerals (phosphorus, calcium, iron, potassium, sodium, zinc, magnesium), vitamin C , thiamine (Vitamin B1), riboflavin (vitamin B2), 2, 3, 6-tri-o-acetyl D lactose units, phenol, tannins, terpenoids ,and saponins, ascorbic acid, carotene,glycosides, volatile oil (Dahiru *et al.,* 2006) and bioflavonoid, pectin-A (Bhuiyan andHoque,2010),flavanoid(quercetin and epicatechin),flavones, isoflavones and anthocyanidins (Beg *et al*.,2016).It is also known to have a high vitamin P (bioflavonoid)

content. *Ziziphus mauritiana* fruit is also known to stimulate bile production (Dahiru *et al.*, 2005), promote circulation, prevent allergies, antibacterial, anti-inflammatory and antioxidant activity are some of the medicinal properties (Beg *et al*., 2016).

The extract of root, and bark of *Ziziphus jujube* exhibited antibacterial activity against 20 bacteria, the leaves of *Ziziphus jujube* have also been shown to have strong anti-allergic activity, chemo tactic (Mishara *etal*.,2011), phagocytic, neuroprotective, a hemo-preventive, and hypotensive and anti-nephritic effect (Beg *et al*., 2016). However, there is paucity of information on the antidepressant effect of *Ziziphus mauritiana*which may be due to the presence of various complex chemical substances of differentcomposition that occur as secondary metabolites.(Najafi *et al*., 2013).

1.3 Statement of Research Problems

Depression is acommon health problem and significant contributor to the global burden of disease that affects people in all communities across the world(WHO, 2012) with steadily increasing incidence and major socio-economic consequences. However, since the knowledge about the underlying pathophysiological principles is still very scanty, depression and other mood disorders are currently diagnosed solely on clinical grounds (Daniela *et al.,* 2010).The high lifetime prevalence of this disabling condition, coupled with limitations in existing medications, make necessary the development of improved therapeutics (Nollet *et a*l.,2013).Additionally, many of these patients who show remission with antidepressant therapy present a relapse of depression upon treatment cessation. However, these modern treatments are expensive, complex and inaccessible for African populations in rural area and medicinal plants have been used for millennia as one of the main sources of therapeutic agents for mankind(Samina *et al.,* 2017).

1.4 Justification

Early adverse experiences such as traumatic life events in childhood result in an increased sensitivity to the effects of stress later in life and influence the individual vulnerability to depression(McEwen 2003).About one in six people in the U.S will succumb to depression at some point during their life span(Kessler *et al.*, 2005), and according to the World Health Organization (WHO), depression is projected to reach second place as leading contributor to the global burden of diseasefollowing cardiac disease by the year 2030(Li *et al*.,2017). The present armamentarium of antidepressants are associated with a number of shortcomings including delayed onset of action, absence of remission about half-life of the patients, side effect profile, drug interaction, late drugefficacy about 3–6 weeks, besides many patients' illness cannot be alleviated (Hua-Cheng *et al.,* 2010). This has necessitated research in to newer compounds with novel mechanism of actions and medicinal plants have been useful sources of important pharmacological drugs (Najafi *et al*., 2013).

Numerous works have implicated the effect of the *Ziziphus mauritiana* on learning and memory (Samina*et al*., 2013), Cognition (Sadiq*et al.*, 2011), Antidiarrheal and Hepatoprotective effects against chronic ethanol-induced hepatotoxicity in rat liver(Dahiru *et al.*, *2006*), Nephrotoxicity assessmentofthehydroethanolic leaf extract of*Ziziphus mauritiana* in mammalians**(**David *et al*., 2017**).**The knowledge and assessments of the biological properties of the *Ziziphus mauritiana* extracts can serve as a source of future drug candidates to patient suffering from depression. The *Ziziphus mauritiana* have been traditionally used for treatment of various complications including insomnia and anxiety (Johanna *etal.,* 2017),and there are many claims about its antidepressant activity.The pertinent question is therefore how does *Ziziphus mauritiana*affect depression since it is common all over the world and it is not expensive,not toxic and easy to use.

However, there is paucity of information on the antidepressant effect of *Ziziphus mauritiana*and it is locally included as part of ingredient used for exorcism in psychological therapy in Northern part of Nigeria. Therefore, this work will attempt to bridge this gap in knowledge.

1.5 Aim

This study determined the antidepressant activity of methanol leaf extract of *Ziziphus mauritiana* in Swiss Albino mice.

1.6 Objectives

i To investigate the phytochemical constituents present in theleaf extract of *Ziziphus mauritiana*

ii To evaluate the effect of Methanol leaf extract of *Ziziphus mauritiana* on Gross Locomotion activity (motor coordination and balance).

iii To determinethe antidepressant activity of Methanol leaf extract of *Ziziphus mauritiana* in acute depression

iv To evaluate the antidepressant activity of Methanol leaf extract of *Ziziphus mauritiana* following BCG(*Bacillus Calmette Guerine*) induced depression modelin mice.

v To evaluate the effect of the Methanol leaf extract of *Ziziphus mauritiana* on febrile response and sickness behaviour.

1.7 Hypothesis

Methanol leaf extract of *Ziziphus mauritiana*has antidepressant activity in Swiss Albino Mice.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Depression

Depression is an affective state of negative mood and low arousal. It is a serious disorder characterized by either a depressed mood or anhedonia (Yang -Hee *et al.,* 2014). The term was derived from the Latin verb *deprimere*, "to press down". From the 14th century, "to depress" meant to subjugate or to bring down in spirits.Depressive disorders, are syndromes characterized by the impairment of mood regulation, most commonly include major depressive disorder and dysthymia, a disorder characterized by chronic low mood (APA, 2013). According to DSM-V the diagnosis of major depressive disorder can be made if a patient has five or more symptoms (depressed mood, loss of interest or pleasure in previously pleasurable activities, significant weight gain or loss, insomnia or hypersomnia, psychomotor agitation or retardation, fatigue, feelings of worthlessness or inappropriate guilt, impaired concentration and recurrent thoughts of death) during the same two-week interval with at least one of the symptoms being either depressed mood or loss of interest or pleasure in activities that were previously pleasurable (WHO, 2012).

Depression is a common and often debilitating illness in old age. Its occurrence is associated with functional impairment, decreased quality of life and increased mortality. It is projected to become the second most common cause of disability by 2020, now regarded as a major public health concern. It is often associated with unemployment and poverty (Strickland *et al*., 2002).Depression is the cause of over two-thirds of the 30,000 reported suicides in the U.S. each year (White House Conference on Mental Health, 1999), for every two homicides committed in

the United States, there are three suicides. The suicide rate for older adults is more than 50% higher than the rate for the nation as a whole. Up to two-thirds of older adult suicides are attributed to untreated or misdiagnosed depression. (American Society on Aging, 1998).

Depression often co-occurs with other illnesses and medical conditions 25% of cancer patients experience depression, 10-27% of post-stroke patients experience depression,1 in 3 heart attack survivors experience depression,1 in 3 HIV(Human immunodeficiency virus) patients , 50% of Parkinson's disease patients,50-75% of eating disorder patients (anorexia and bulimia) 27% of individuals with substance abuse disorders,both alcohol and other substances,8.5-27% of persons with diabetes experience depression may experience depression(National Institute of Mental Health, 2002).

Depression is comorbid with numerous inflammatory diseases such as aging, obesity, and cardiovascular diseases (example atherosclerosis, congestive heart failure), rheumatoid arthritis), and also diseases of the central nervous system such as Alzheimer's, Parkinson's or human immunodeficiency virus (HIV) diseases (O'Connnor *et al*.,2009). Major depression shares etiologies with inflammatory disease and some postulate that depression is an inflammatory disease. Inflammatory factors such as cytokines have been extensively correlated with depressive disorders in both clinical and experimental models (Marcus, 2013). A number of vulnerability factors may predispose towards depression by enhancing inflammatory reactions, e.g. lower peptidase activities (dipeptidyl-peptidase IV, DPP IV), lower omega-3 polyunsaturated levels and an increased gut permeability (leaky gut). The cytokine hypothesis considers that external, e.g. psychosocial stressors, and internal stressors, e.g. organic inflammatory disorders or conditions, such as the postpartum period, may trigger depression via inflammatory processes. Most if not all antidepressants have specific anti-inflammatory effects, while restoration of

decreased neurogenesis, which may be induced by inflammatory processes, may be related to the therapeutic efficacy of antidepressant treatments (Yang *etal.*, 2016).

Major depressive disorder in elderly persons is associated with an increased number of suicide attempts and increased lethality (Gureje, 2007). In fact, suicide in the elderly population is twice as common as in the general population, interestingly, MDD is one of the major causes of suicide in subjects with late-life depression because approximately 80% of people who commit suicide show depressive symptoms (Kupfer *et al.*,2012). Clinical Studies showed elderly patients with depression are at risk for suicidal ideation and found that contemporaneous severity of depression was the most important determinant of suicidal ideation over time (Dwivedi, 2013). Major depressive disorder, a common disease, results from a complex interplay of genes and environmental risk factors as well as other common multifactorial diseases such cancer and diabetes mellitus and cardiovascular disease. Environmental factors, such as pre-natal factors, loss, deprivation, grief, stress, natural disasters, war, social support systems, nutrition, exercise, drug effects, and medical illness, stress, have been linked to major depressive disorder (Chikako and Kouichi, 2009). As for genetic factors, only minor susceptibility genes have been reliably identified.Major depressive disorder is caused by multiple genes and does not follow Mendel's laws of inheritance and is currently the leading cause of disease burden in high income countries (Bennet, 2014).

Prevalence of depression was 4.4% globally, 5% in North east, sub-Saharan Africa, Eastern Europe & Middle East (Sophie, 2013).Women suffer 1.5-3 fold higher rate of depression than males, 26.2% of elders in Ibadan-Nigeria (Gureje *et al.*, 2007).Middle East, North Africa suffer the world highest depression rate, in case of the North, conflict in the region increases the prevalence due to war trauma and stress (Sophie, 2013). The overall prevalence of depression

was found to be 5.2% in Oyo State, Nigeria. Depression was more prevalent among women than men and among adolescents. Furthermore, depression was more common in the rural areas than in the urban areasof Ibadan, Nigeria (Amoran *et al.*, 2007). Another study conducted in Teaching Hospitals of 3 South East states in Nigeria showed that the prevalence of depression was 17.3% in resident doctors and 1.3% in non-resident doctors , 19.6% in female resident doctors and 16.3% in male resident doctors,15.9% married and 20.4% single resident doctors had diagnosis of depression (Aguocha *et al*.,2015). The study of Abiodun (2006) revealed that the current (2 weeks) prevalence of MDD among Nigerian college students with alcohol dependence is 23.8%; alcohol abuse 17.2% and hazardous use is 12.4%.

Recent epidemiological studies indicate that severe forms of depression affect 2-5% of the population worldwide, and up to 20% are affected by milder forms of the disease (Kessler *et al*., 2007). Moreover, depressive patients have a 2-4 fold increased risk of developing cardiovascular diseases and 10-15% of individuals with major depression commit suicide (Chikako and Kouichi, 2009).Women may suffer from unique forms of depression related to their unique biology and life experiences and may be more likely to seek treatmentthan men. Hormonal factors may contribute to the increased rate of depression in women, particularly such factors as premenstrual changes, pregnancy, miscarriage, postpartum period, pre menopause, and menopause (Schmidt, 2005). Many women face additional stresses, such as responsibilities at work and home, single parenthood, and caring for children and aging parents. Women suffer from depression more often and attempt suicide more frequently, men are more successful in their suicide attempt. Depressive disorders are most common to observe in urban than in rural population and the prevalence is in groups with stronger socioeconomic factors i.e. homelessness (Gureje *etal*.,2007).

2.2 Subtypes of Major Depressive Disorder

The DSM V recognizes five further subtypes of MDD, called specifies, in addition to noting the length, severity and presence of psychotic features:

2.2.1 Melancholic depression

It is characterized by a loss of pleasure in most or all activities, a failure of reactivity to pleasurable stimuli, a quality of depressed mood more pronounced than that of grief or loss, a worsening of symptoms in the morning hours, early-morning waking, psychomotor retardation, excessive weight loss (not to be confused with anorexia nervosa), or excessive guilt (Douglas *et al.*, 2015).

2.2.2 Atypical depression

It is characterized by mood reactivity (paradoxical anhedonia) and positivity, significant weight gain or increased appetite (comfort eating), excessive sleep or sleepiness (hypersomnia), a sensation of heaviness in limbs known as leaden paralysis, and significant social impairment as a consequence of hypersensitivity to perceived interpersonal rejection(Abiodun, 2006).

2.2.3. Catatonic depression

It is a rare and severe form of major depression involving disturbances of motor behaviour and other symptoms, the person is mute and almost stuporous, and either remains immobile or

exhibits purposeless or even bizarre movements. Catatonic symptoms also occur in schizophrenia or in manic episodes, or may be caused by neuroleptic malignant syndrome. (Chikako and Kouichi 2009).

2.2.4.Postpartum depression

It is mental and behavioral disorders associated with the puerperium, not elsewhere classified, refers to the intense, sustained and sometimes disabling depression experienced by women after giving birth (Abiodun, 2006). Postpartum depression has an incidence rate of 10–15% among newmothers(WHO, 2012). The DSM V mandates that, in order to qualify as postpartum depression, onset must occur within one month of delivery. It has been said that postpartum depression can last as long as three months (Schmidt, 2005).Postpartum mood changes can range from transient "blues" immediately following childbirth to an episode of major depression and even to severe, incapacitating, psychotic depression. Studies suggest that women who experience major depression after childbirth very often have had prior depressive episodes even though they may not have been diagnosed or treated. (National Institute of Mental Health, 1999).

2.2.5Seasonal affective disorder (SAD)

It is a form of depression in which depressive episodes come on in the autumn or winter, and resolve in spring. The diagnosis is made if at least two episodes have occurred in colder months with none at other times, over a two-year period or longer (Abiodun, 2006).

2.3 Causes of Depression

The complexity and heterogeneity of depression make it difficult to identify a single underlying abnormality, and suggest that there are multiple causes of depression (Bun Hee and Yong, 2010). Adversity in childhood, such as grief, neglect, mental abuse, and unequal parental treatment of sibling, physical or sexual abuse in particular significantly increases the likelihood of experiencing depression over the life course (Pillemer *et al*., 2010).Life events and changes that may precipitate depressed mood include childbirth, menopause, financial difficulties, job problems, a medical diagnosis, bullying, loss of a relation, natural disasters, social isolation, relationship troubles, jealousy, separation, catastrophic injury and social rejection (Lindert et *al.*, 2014).

Biological causes of depression vary but may relate to malnutrition, heredity, hormones, seasons, stress, illness, drug or alcohol use, neurotransmitter malfunction, long-term exposure to dampness and mold, back injury, and to aerosol exposure (Nesse, 2000).The cytokine hypothesis considers that external stressors example psychosocial stressors, and internal stressors, e.g. organic inflammatory disorders or conditions, such as the postpartum period, may trigger depression via inflammatory processes (Maes *et al*., 2009).

2.4 Neurobiology of Depression

Depression is a common psychiatric disorder, with diverse symptoms and high comorbidity with other brain dysfunctions. Due to this complexity, little is known about the neural and genetic mechanisms involved in depressive pathogenesis (Bergner *et al*., 2010). Major depression is characterized by different neurobiological alterations as well as structural and functional changes in brain structures, such as the prefrontal cortex (PFC) and the hippocampus, which play a crucial role in emotion, mood, and cognition (Kang *et al*.,2012), it is also associated with

impaired neuronal plasticity. Suicidal behavior can be a consequence of severely impaired neuronal plasticity in the brain (Dwevidi,*etal*., 2013).However studies over the past 15 to 20 years have continued to build on evidence that stress and depression are associated with atrophy and loss of neurons and glia, and there is even reduced volume of key limbic and cortical brain regions in depression (Alexopolous, 2002). Preclinical and clinical studies demonstrate that reductions of the total volume of neurons and neuronal loss occur in stress and depression in the adult hippocampus. These hippocampal alterations can be reversed by chronic antidepressant treatment (Bun Hee and Yong, 2010).

MDD is a complex pathology, associated with monoamines and also non-monoaminergic neurotransmitter systems like GABA, glutamate, peptides, and cannabinoids), with alterations in signaling pathways (Brain derived neurotropic factor (BDNF), tropomyosin-related kinase B receptor, extracellular signal regulated kinase,and Akt pathways) and with hormonal dysregulation (example, of the hypothalamic–pituitary adrenal (HPA) axis) (Eric *etal.*, 2016). Furthermore, in recent years, epigenetic modifications (histone acetylation and methylation), altered glial function (example astrocyte deficit), inflammation (excess of proinflammatory cytokines), and decreased neural plasticity (hippocampal neurogenesis, hippocampal, and cortical synaptogenesis) have also been described (Willner *et al*., 2012). The pathogenesis of MDD involves altered neural plasticity, resulting in the inability of the brain to make appropriate adaptive responses to environmental stimuli. The affective frontal-limbic circuitry, including the prefrontal cortices, the cingulate cortex, and several limbic structures (including the hippocampus), are highly involved in mediating these adaptive responses (Alexopolous, 2002). Several lines of evidence demonstrate that these regions show structural and functional alterations in MDD. These include a reduction in cell number, density, cell body size, neuronal

and glial density in frontal cortical or hippocampal brain areas, and parahippocampal cortex and cortical/laminar thickness (Leuner and Shors, 2012).

In addition, studies in MDD subjects show changes in synaptic circuitry in anterior limbic cortex, abnormal dorsolateral prefrontal cortical activity, impaired synaptic connectivity between the frontal lobe and other brain regions, neuronal atrophy, a decreased volume of the hippocampus, a decreased number of neurons and glia in cortical areas, and spatial cognition deficits(Sexton *etal*.,2013). In addition, changes in the number and shape of dendritic spines, changes in the primary location of synapse formation, altered dendritic morphological characteristics of neurons in the hippocampus, and a decrease in length and number of apical dendrites have been reported during stress in mice and rats (Wohleb *et al*., 2016). Neurogenesis is consistently boosted by antidepressants in animal models only when animals are stressed. Ablation of neurogenesis in animal models impairs cognitive functions relevant to depression, but only a minority of studies found that ablation causes depression or anxiety (Miller and Hen, 2015).

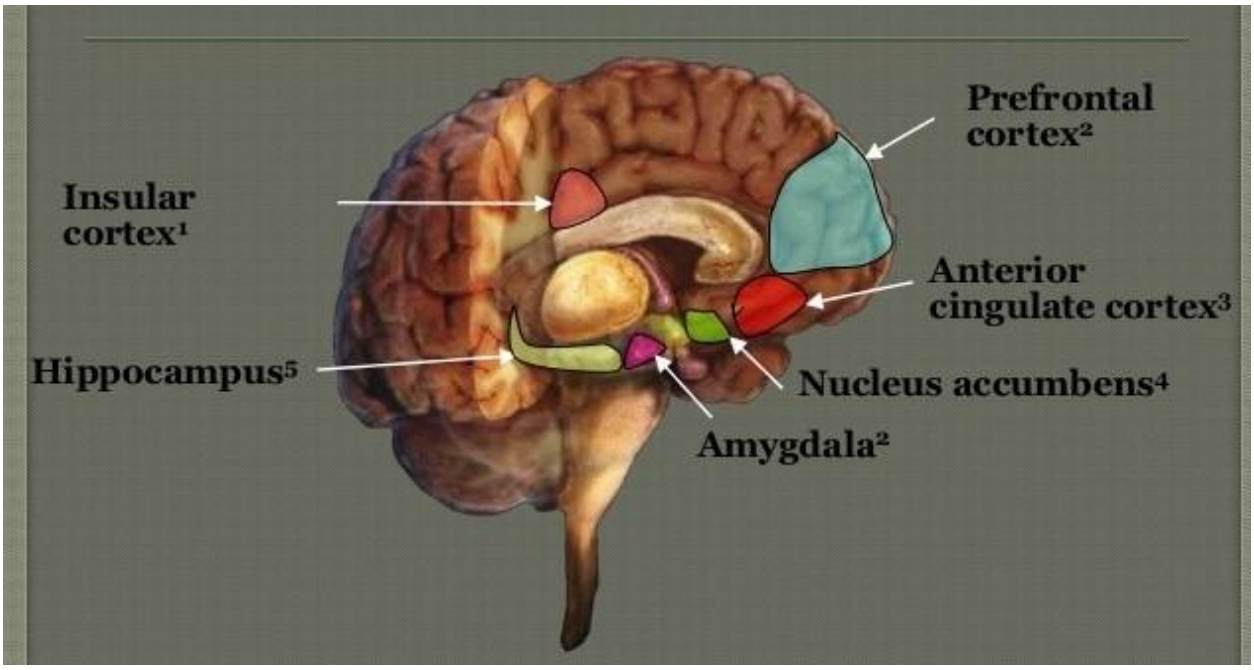


Plate II Areas in the brain implicated in Depression (Adapted from Kennedy *et al* 2006)

2.5 Pathophysiology of Depression

The underlying pathophysiology of depression remains unclear despite the seriousness and prevalence of this disorder (Dean and Keshavan, 2017). Clinical symptoms of depression manifest at psychological, physiological, and behavioural levels and include changes in appetite and sleeping patterns, sad or irritable mood, psychomotor agitation, fatigue, anhedonia, poor concentration, feelings of guilt, and recurrent thoughts of suicide or death (Bergner *et al*.,2010). There are several different endophenotypes of depression with distinct pathophysiological mechanisms, some possible pathophysiological mechanisms of depression include altered neurotransmission, HPA axis abnormalities involved in chronic stress, inflammation, reduced neuroplasticity, and network dysfunction. All of these proposed mechanisms are integrally related and interact bidirectionally. In addition, psychological factors have been shown to have a direct effect on neurodevelopment, causing a biological predisposition to depression, while biological factors can lead to psychological pathology as well (Dean and Keshavan, 2017).

2.6 Hypotheses of Depression

2.6.1. Neurodegeneration and Serotonin /Cytokines hypotheses of depression

There are different theories and hypotheses related to the aetiology of depression.The interaction between brain 5-HT level and the activity of its autoreceptors play a role in mood changes and depression. In major depression, activation of the inflammatory response system (IRS) and increased concentrations of proinflammatory cytokines, prostaglandin E2 and negative immuno-regulatory cytokines in peripheral blood have been reported (Myint and Kim, 2003). Recently,

pro-inflammatory cytokines have been found to have profound effects on the metabolism of brain serotonin through the enzyme indoleamine-2,3-dioxygenase (IDO) that metabolizes tryptophan, the precursor of 5-HT to neurodegenerative quinolinate and neuroprotective kynurenate. The cytokine–serotonin interaction that leads to the challenge between quinolinate and kynurenate in the brain explains the neurodegeneration hypothesis of depression (O'Connor*et al*., 2009).

The serotonin hypothesis of depression postulated that decrease in serotonin or serotoninergic transmission causes depression. Activation of IDO by proinflammatory cytokines alters serotoninergic and glutamatergic neurotransmission. Since tryptophan is the limiting factor for the synthesis of serotonin, decreased circulating tryptophan concentrations, as occurs in patients undergoing immunotherapy, have the potential to negatively impact serotoninergic neurotransmission. IDO is also expressed in brain endothelial cells, perivascular macrophages, astrocytes and microglia, so that fluctuations in its enzymatic activity can alter brain tryptophan metabolism which may precipitate depressive behaviour. When IDO is activated in conditions of chronic inflammation, its degree of activation is correlated to the intensity of depressive symptoms, as observed in cancer patients chronically treated with IFN-α (O'Connor *et al.*, 2009).Most prescribed antidepressants increase serotonin (5-HT) availability at the synapse through reuptake inhibition. Therefore, much focus has been placed on examining the neurobiology of serotonergic systems as a mechanism of treatment resistant depression (Coplan *et al*., 2014).

2.6.2. Glutamate hypothesis of depression

The glutamatergic system is a primary mediator of psychiatric pathology and, potentially, also a final common pathway for the therapeutic action of antidepressant agents. Pathophysiology of

depression is associated with dysfunction of glutamatergic system (Blier, 2016). Drugs targeting glutamate system may have rapid and sustained antidepressant effects. A large number of clinical studies suggest that pathophysiology of depression is associated with dysfunction of the predominant glutamatergic system, malfunction in the mechanisms regulating clearance and metabolism of glutamate, and cytoarchitectural /morphological maladaptive changes in a number of brain areas mediating cognitive–emotional behaviours. Concurrently, a wealth of data from animal models have shown that different types of environmental stress enhance glutamate release/transmission in limbic/cortical areas and exert powerful structural effects, inducing dendritic remodeling, reduction of synapses and possibly volumetric reductions resembling those observed in depressed patients(Gerard *et al*., 2012).

2.6.3.Cholinergic hypothesis of depression

The cholinergic hypothesis of depression postulates that depression may be due to an over activity or hypersensitivity of the cholinergic system over the adrenergic system (Van Enkhuizen *et al.,* 2014). Several investigators have suggested that decreasing acetylcholine neurotransmission, through muscarinic or nicotinic acetylcholine receptor blockade, may be an effective novel treatment for depression and that traditional antidepressants may act in part via acetylcholine receptor antagonism (Drevets *et al*., 2013; Jaffe *et al*., 2013).

2.6.4. Dopaminergic hypothesis

The dopaminergic hypothesis of depression postulated that hypodopaminergic state is thought to produce anhedonia and drugs that increase dopaminergic activity in the mesolimbic dopamine system may reverse the anhedonia associated with depression (Nemeroff, 2007).

2.6.5. Brain-Derived Neurotropic Factor (BDNF) hypothesis

The BDNF hypothesis of depression postulates that a reduction in BDNF is directly involved in the pathophysiology of depression, while anti-depressant mediated restoration of BDNF is responsible for the alleviation of the depressive state (Bus *etal*., 2011).

Brain-derived neurotropic factor (BDNF) is a member of the neurotrophin family of growth factors, which are related to the canonical Nerve Growth Factor, It is a protein in humans, that is encoded by the BDNFgene. Neurotrophic factors are found in the brain and the periphery. Brain derived neurotrophic factor (BDNF) is widely expressed throughout the central nervous system (CNS) (Duman *etal.*,2012). It is involved in proliferation, differentiation, survival and death of neuronal and non-neuronal cells in the developing and adult CNS. BDNF is involved in activity-dependent neuronal plasticity, such as learning and memory (Duman and Monteggia 2006), and has been shown to modulate the development and function of synapses in various systems, ranging from the neuromuscular junction to the cortex. In the hippocampus, substantial evidence suggests that BDNF facilitates both early-phase and late-phase long term potentiation (LTP) (E-LTP and L-LTP). Experiments using gene-knockout mice have shown that BDNF is required for LTP (Bun-Hee and Yong, 2010).

BDNF influences a variety of functions including: preventing death of existing brain cells, inducing the growth of new neurons (neurogenesis) and synapses, and supporting cognitive function. Low levels of BDNF are often problematic and have been linked to Alzheimer's disease, accelerated aging, poor neural development, neurotransmitter dysfunction, obesity, and depression(Grove, 2007). BDNF is one of many neurotrophins in the brain that helps stimulate as well as manage the process of neurogenesis. Although there are a variety of neurotrophins at work in the brain, BDNF is regarded as being among the most active as well as universally important. Therefore, maintaining satisfactory levels of BDNF results in optimal

neurotransmission and potentially prevents a myriad of physical as well as mental diseases (Duman*etal*., 2012).

2.6.6 Circadian rhythm hypothesis

This hypothesis stated that the depression may be related to abnormalities in the biological clock or circadian rhythm, for example, rapid eye movement (REM) sleep(the stage in which dreaming occurs) it might be quick to arrive and intense in depressed people. REM sleep depends on decreased serotonin levels in the brain stem and is impaired by compounds such as antidepressants that increase serotoninergic tone in brain stem structures (Adrien,2003). However, the serotonergic system is least active during sleep and most active during wakefulness, prolong wakefulness due to sleep deprivation activates serotoninergic neuron leading to processes similar to the therapeutic effect antidepressant such as Selective serotonin reuptake inhibitor (SSRIs).Depressed individuals can exhibit a significant lift in mood after a night of sleep deprivation. SSRIs may directly depend on the increase of central serotoninergic neurotransmission for their therapeutic effect the same system that impacts cycles of sleep and wakefulness(Carlson,2013).

2.7 Overview of Antidepressants

Antidepressants, such as selective serotonin reuptake inhibitors (SSRIs), may alleviate depressive symptoms but also leave residual symptoms. The mechanism of action of SSRIs increases serotonin (5-HT) activity but decreases norepinephrine (NE) and dopamine (DA) activity (Blier, 2016). The current standard of care for major depressive disorder is pharmacological treatments that modulate monoamines. First generation antidepressants, including monoamine oxidase inhibitors(example,Tranylcypramine(MAO selective),Phenelzine(MAO selective),Moclobemide(MAO non selective), and tricyclic antidepressants (example, Desipramine (NRI), Nortriptyline (NRI),Reboxetine (NRI), Amitriptyline (SRI+NRI), Imipramine (SRI+NRI),Venlafaxine (NRI+SRI),Milnacipran (NRI+SRI),Duloxetine(NRI+SRI)were effective in treating depression but caused a wide range of side effects. Second generation Antidepressants, including Selective Serotonin Reuptake Inhibitors {(Fluoxetine(SRI),Paroxetine (SRI),Sertraline (SRI)Fluvoxamine(SRI),Citalopram(SRI),Escitalopram(SRI)}.Selective Norepinephrine reuptake inhibitors (NRI), Serotonin/Norepinephrine Reuptake Inhibitors (SNRI), and Dopamine/Norepinephrine reuptake inhibitors(example Bupropion (NRI+DRI) (Caldarone *et al*., 2015), improved the side effect profile, but are still sub-optimal due to two major limitations. First, there is a delayed response between the start of treatment and the onset of beneficial effects, a lag that can often take several weeks; second, there is often an inadequate response to the pharmacological treatment, referred to as treatment resistance, with only approximately one third of patients achieving remission after treatment with a standard SSRI (Trivedi *et al.,* 2006).

Therefore, Serotonin reuptake inhibitor (SRI) prevents serotonin reuptake by inhibition of the serotonin transporter, Norepinephrine reuptake inhibitor (NRI) prevents Norepinephrine reuptake

by inhibition of the Norepinephrine transporter.Dopamine reuptake inhibitor (DRI): prevents Dopamine reuptake by inhibition of the Dopamine transporter(Caldarone *et al*., (2015).

2.7.1 CurrentAntidepressant

A unique fast neuroprotective and antidepressant treatment effect has been observed by ketamine, which acts via the glutamatergic system. Based on glutamatergic signaling, a number of therapeutic drugs such as ketamine, memantine, amantadine, tianeptine, pioglitazone, riluzole, lamotrigine, magnesium,zinc,guanosine,adenosine aniracetam, traxoprodil, D-serine, dextromethorphan, sarcosine, scopolamine, pomaglumetad methionil, 1 aminocyclopropanecarboxylic acid, all of which target the glutamatergic system might open up a promising new territory for the development of drugs to meet the needs of patients with major depression (Deutschenbaur *et al.*,2016).

2.8 Antidepressant and *Ziziphus mauritiana*

Research has devoted much of its attention for curbing depressionand many drugs have been developed and are currently prescribed to treat this pathology. Yet, many patients are refractory to the available therapeutic drugs, which mainly act by increasing the levels of the monoamines serotonin and noradrenaline in the synaptic cleft (Strekalova *etal*.,2006). Even in the cases where antidepressants are effective, it is usually observed after a delay of a few weeks between the onset of treatment and remission of the clinical symptoms. Additionally, many of these patients who show remission with antidepressant therapy present a relapse of depression upon treatment cessation. Thus research has focused on other possible molecular targets, besides monoamines, underlying depression. Both basic and clinical evidence indicates that depression is associated with several structural and neurochemical changes (Eric *etal*., 2016). However, these modern

treatments are expensive, complex and inaccessible for African populations in rural area. Medicinal plants have been used for millennia as one of the main sources of therapeutic agents for mankind.Even today, a majority of the world's population relies on plants as a primary source of traditional medicine (Kinghorn *et al.*,2011). The knowledge and assessments of the biological properties of extracts from plants can serve as a source of future drug candidates in many areas of health.(Samina *et al.,* 2017).The discovery of new drug leads from medicinal plants may be aided by ethno pharmacology (Abdur Rauf *et al*.,2016).In recent years, there has been a phenomenal rise in the interest of scientific community to explore the Pharmacological actions or to confirm the veracity of claims made about herbs (Une *et al.*, 2012).

*Ziziphus mauritiana*is a tropical or subtropical fruit tree widely distributed in many Asian countries such as Afghanistan, Bhutan, India, Indonesia, Malaysia, Myanmar, Nepal, Sri Lanka, Vietnam, Africa, Australia, and Thailand(Aye *et al.*,2013).The seeds of *Z. mauritiana*, a species close to *Z. jujuba* Mill., have been reported as anticancer, antidiabetic, and hypoglycemic agents (Bhatia and Mishara.,2010), used to treat insomnia and reduce the body temperature and sweat (Jarald *et al*.,2009). The seeds of *Ziziphus mauritiana*have been traditionally used for treatment of various complications including insomnia and anxiety. They are popularly used as sedative and hypnotic drugs in China, Korea, Myanmar, Vietnam, and other Asian countries.*Ziziphus* seeds contain large amounts of fatty oil and proteins, sterols, and triterpenoid compounds (betulin and betulinic acid) and also contain a large amount of vitamin C. The various species of genus *Ziziphus* showed antipyretic, antidiuretic, anti-inflammatory, and analgesic activities. The plant is used to cure wide variety of diseases such as fever, ulcer, asthma, depression, anxiety, and inflammation, and is enriched with biological and phytochemical properties (Mahajan and

Chopda, 2009). However, no scientific proofon antidepressant activity of Methanol leaf extract*Ziziphus mauritiana* was reported.

2.9*Bacillus Calmette-Guérin* (BCG)

Bacillus Calmette-Guérin (BCG) is an attenuated form of *Mycobacterium bovis* that is used as a vaccine for tuberculosis incorporated into childhood vaccination schedules globally. It is administered to neonates and children worldwide (Moreau *et al.,* 2008). In addition to the prevention of tuberculosis, BCG has been used as an immune modulator and even as immunotherapy in certain non-infectious diseases. Furthermore, recent clinical and experimental studies have revealed that BCG has a neuroprotective role in several central nervous system (CNS) (Yang *et al*., 2016).Early-life immune activation has been well-established in several countries outside the United States (Brewer, 2000). Intraperitoneal administration of BCG to mice is rapidly followed by long-lasting mycobacterial dissemination in all organs (particularly the lungs) except the brain and by a drastic increase in circulating IFN-γ, a key cytokine for the activation of IDO(Moreau *et al*., 2005).It has been shown to chronically activate both lung and brain indoleamine 2,3-dioxygenase (IDO), a tryptophan-catabolizing enzyme that mediates the occurrence of depressive-like behavior following acute innate immune system activation (Moreau *et al*., 2008). IDO is also expressed in brain endothelial cells, perivascular macrophages, astrocytes and microglia, its activation by proinflammatory cytokines alters serotoninergic and glutamatergic neurotransmission (O'Connor *et al*., 2009).

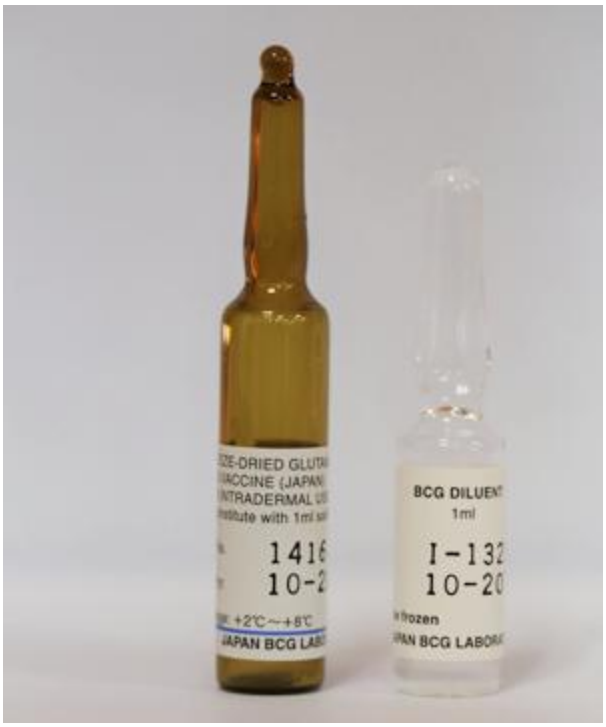


Plate III BCG Vaccine vial (Snapped by the Candidate 2018)

2.9.1 *Bacillus Calmette-Guérin (*BCG) as a model of depression

BCG inoculation induced an acute episode of sickness (approximately 5 days) that was followed by development of delayed depressive-like behaviors lasting over several weeks. Transient body weight loss, reduction of motor activity and the febrile response via chronic activation of the immune system (Moreau *et al*., 2008). The same effect happened in laboratory animals, through the administration of the cytokine inducer lipopolysaccharide (LPS) which induced depressive-like behavior, through acute activation of the peripheral innate immune system as measured by increased immobility in the forced swim test and tail suspension test, decreased consumption of a sweetened solution and a suppression of sexual behavior that can be attenuated by chronic antidepressant administration (O'Connor *et al.*,2009).The mice were inoculated with BCG once

throughout the study.Moreover, the effect of Methanol leaf extract of *Ziziphus mauritiana* on febrile response and sickness behavior symptoms was also investigated by measuring body temperature and body weight respectively.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Collection

Fresh leaves of *Ziziphus mauritiana* werecollected from Tudun wada, Zaria, Kaduna state Nigeria. The sample was identified by Mallam Sunusi Namadi at Herbarium section of the Department of Botany, Ahmadu Bello University,Zaria, where voucher specimen number 90123 was deposited.

3.1.1 Plant extraction

Fresh leaves of *Ziziphus mauritiana*, were air dried under shade and subsequently size-reduced. Methanol extract of the leaves were obtained by a modification of Nmila *et al*.(2000) where50g of dried ground materials was separately extracted with 500 ml of methanol using maceration

method, then the extract was put in sterile bottles containing 95% methanol and was kept in room temperature. The methanol extract was used to pre-treat the mice 1 hour before subjecting it to test (Najafi *et al.*, 2013).

An established antidepressant drug Imipramine (SSRI) 20mg/kg was dissolved in distilled water and administered orally to animals 1 hour before the test.

Thefreeze dried BCG (*Bacillus Calmette-Guerin*) vaccine was diluted with its 1ml diluent properly and administered intraperitoneally (*i.p*) in a volume of 200 µl/mouse.

3.2 Materials

A total of about 15 Stainless Steel Cages were used to house the mice, Saw Dust was used to bed the cages,grower mesh was used as a feeds, distilled water was used as a control in the study, Weighing Balance was used to weigh the mice's body weight, Beaker was used to measure the sucrose and its intake level, BCG Vaccine (Manufacture by BB- NCIPD Ltd., Sofia, Bulgaria, InterVax Ltd., Canada) was used to induced depressive like behaviour, Cotton Wool and Methylated spirit was used to clean the apparatus between the test,, Heater, Bucket, Feeder, Stop Watch, Masking Tape was used in suspending the mice's tails, Marker was used to mark the animals in to groups,rectalThermometer was used to record the mice's body

temperature,Sucrose,Digital Cameras (JVC Micro HDD, Hard disc Cam Decorder Everio 30GB,35xOptical zoom/AF,Hybrie:LY36228-001A, F=2.2,Company Name:Victor Company of Japan, Ltd. and Samsung (ES95) N363 F2.5 bright lens 5x optical zoom,6.2 Mega Pixels DC=5.0V,S/N AZZQCNNOD-00EKJ) was used to record the activity of the mice subjected to the tests, 2ml syringes was used to inject BCG in to the mice , oral cannula was used to administer the extract orally to the mice, mortar and pestle was used to mix the extract and drug, electronic weighing machine(Salter,HoMedics Group Ltd.Production no.1066-1011-03)was used to measure the extract

3.3Animals and their Management

Apparently healthy One Hundred and Eight (108) mice weighing 19 –22 g was used for this study. The animals were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria.The mice were kept in well-ventilated cages in the animal house of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, with 24 hours photoperiod. They were allowed to acclimatize to the housing and experimental conditions for two weeks. They were given access to standard animal feeds and tap water *ad libitum*.

Mice were divided into two major groups namely: acute Antidepressant studies and chronic antidepressant studies.

3.4 Acute Toxicity Study

The acute toxicity study was carried out based on the method described by Lorke (1983). Briefly, the method was done in two phases, in the first phase, three groups of three mice each were treated orally with 10, 100 and 1000mg/kg body weight and observed for signs and symptoms of toxicity and death for 24 hours. In the second phase, four groups each containing one mouse was treated with four more specific doses of the extract (1200, 1600, 2900 and 5000 mg/kg). The LD_{50} was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

After oral administration of extract, animals were also observed individually for signs of toxicity such as behavioral changes including convulsion, difficulty in movement tremor, salivation, writhing and death for 24 hours. The doses of the extract were chosen based on the estimated LD_{50} according to Vongtau *et al.* (2004) who stated that the dose should be 30% of the determined $LD_{50.}$

3.5 Acute Antidepressant Studies

The mice was randomly assigned into the following experimental groups, each group consisting of 6 animals each:

Group 1: Control group, mice in this group were administered distilled water (10 ml/kg) orally.

Group 2:Mice in this group were administered 200mg/kg Methanol leaf extract *Z.mauritiana* orally

Group 3: Mice in this group were administered 100mg/kg Methanol leaf extract *Z.mauritiana* orally

Group 4: Mice in this group were administered 50 mg/kg Methanol leaf extract *Z.mauritiana* orally

Group 5: Micein this group were administered 25 mg/kg Methanol leaf extract *Z.mauritiana* orally

Group 6: Mice in this group were administered 20 mg/kg Imipramine orally (Shehu *etal*., 2017).

In acutestudy,beam walking assay test, Tail Suspension Test, Open Field Test, Forced Swim Test, and Novel Object Recognition Test were assessed.

3.6 Chronic Antidepressant Study

The mice was randomly assigned into the following experimental groups, each group consisting of 6 animals:

Group 1: Control group, mice in this group were administered distilled water only (10ml/kg) orally.

Group 2: Mice in this group were administered 0.2mg/kg BCG (*i.p*) + distilled water orally.

Group 3: Mice in this group were administered 0.2mg/kg BCG (*i.p*) + 200mg/kg of the methanol leaf extract ZZM orally.

Group 4: Mice in this group were administered 0.2mg/kg BCG (*i.p*) + 100mg/kg of the methanol leaf extract ZZM orally.

Group 5: Mice in this group were administered 0.2mg/kg BCG (*i.p*) + 50mg/kg of the methanol leaf extract ZZM orally.

Group 6: Mice in this group were administered 0.2mg/kg BCG (*i.p*) + 25mg/kg of the methanol leaf extract ZZM orally.

Group 7: Mice in this group were administered 0.2mg/kg BCG (*i.p*) + 20mg/kg Imipramine orally.

Mice's body weight and temperature were assessed first followed by locomotory activity and sucrose preference.

3.6.1 Induction of depression using BCG (*Bacillus Calmette-Guerin*).

The method of Moreau *et al*. (2009) was adopted. On the inoculation day, the stock solution of BCG(*Bacillus Calmette-Guerin*) was diluted with its diluent, and administered intraperitoneally *(i.p*) in a volume of 200 µl/mouse. The dose of BCG (0.2mg/kg) was selected on the basis of its ability to reliably increase peripheral and central IDO activity (Moreau *et al*., 2005). Before the BCG induction, mice's body weight were taken for 4 days and for 7 consecutive days after. All experimental sub-groups were matched for body weight before treatment, mice's body temperature (rectal temperature) were also taken 1 hour before the induction and each hour after. On the day of assessment, locomotory and rearing activities were measured first, followed by the Tail Suspension Test and Sucrose Preference Test.

3.7. Neuro Behavioural Models

3.7.1 Sucrose preference test

Before BCG inoculation, the mice were given 1% sucrose solution for 24 h. Then, both sucrose solution and fresh water were made accessible to the mice for another 24 h. After been deprived of drinking for 23 h, the mice were given choice between two bottles, one with 1% sucrose solution and another with fresh water for 1h again. After this sucrose consumption training phase, the animals were randomly divided into 7 experimental groups (6 mice per group).Sucrose preference test was conducted following BCG Inoculation at 1^{st},7^{th},and 21^{st} week post inoculation. Sucrose preference was calculated as sucrose preference (%) = Sucrose intake (ml)/ [sucrose intake (ml) +water intake (ml)] ×100%. The treatment protocol were adopted from (Li *et al*., 2013). The consumption of sucrose and water were estimated simultaneously in control and experimental groups, a decrease in sucrose preference and intake was taken as a criterion for anhedonia and depression (Tatyana *et al.*, 2004).



Plate IV Sucrose Preference Test Cage (Snapped by the Candidate, 2016)

3.7.2. Forced-Swimming Test (FST)

Each mouse was placed in a plexiglass cylinder tank of 40cm height and 18cm width filled with 15cm water at 25℃. The total duration immobility was measured over 5 minutes. A mouse was considered immobile whenever it remained floating passively in the water in a slightlyhunched but upright position with its nose just abovethe surface (Porsolt, 2000). In this test, immobility were interpreted as a passive stress-coping strategy or depression-like behavior (behavioral despair) (Porslt 1977, Porsolt *et al.*, 1987, Can *et al*., 2012).



Plate V Force Swimming Test Apparatus (Snapped by the Candidate, 2017)

3.7.3 Tail Suspension Test (TST)

The Tail Suspension Test (TST) was developed bySteru *et al.*(1987)andCan *et al*.(2012). It is a test best validated for the evaluation of antidepressant efficacy of drugs, but also used to evaluate the effects of environmental, neurobiological, and genetic manipulations (Lad *et al*., 2007). In contrast to the forced swim test, in the TST there is no risk of hypothermia due to submersion in water (Porsolt *et al.*, 1987).

Mice were hung by threading their tails through a 1cm hole in a board measuring 10 cm by 20 cm. The board was suspended from a stand 30 cm in height. Tails were secured with lab tape, approximately 2 cm from the base of the tail to the opposite side of the board. Latency to immobility, number of immobile segments, and total time spent immobile were recorded during the 6 min test. Typically, the suspended rodents perform immediately escape-like behaviors, followed by developing an immobile posture. If antidepressants are given prior to the test, the subjects will be engaged in escape-directed behaviors for longer periods of time than after saline treatment, exhibiting a decrease in duration of immobility (Cryan *et al.*, 2005).



Plate VI Tail Suspension Test Apparatus(Snapped by the Candidate, 2016)

3.7.4 Open Field Test

The Open field test (OFT) is a simple sensorimotor test developed by Calvin S. Hall to test emotionality of rodents. It is commonly used qualitative and quantitative measure to determine general activity levels, gross locomotory activity, and exploration habits in rodent models of CNS disorders (Careau *et al.*, 2012). Assessment takes place in a square, white Plexiglas box. The open field is an arena with walls to prevent escape. Commonly, the field is marked with a grid and square crossings. Mice were carried to the test room in their home cages and were handled by the base of their tails at all times. Mice were placed into the center or one of the four corners of the open field and allowed to explore the apparatus for 5 minutes. After the 5 minute test, mice were returned in their home cages and the open field apparatus was cleaned with 70 % ethyl alcohol and permitted to dry between the tests. The number of line crosses and the frequency of rearing were used as measures of locomotory activity .The OFT is also commonly used as a mechanism to assess the sedative, toxic, or stimulant effects of compounds, The OFT is

also often used to assess anxiety by including additional measures of defecation, time spent in the center of the field, and the first few minutes of activity (Todd *et al.,* 2009).



Plate VII Open Field Test (Snapped by the Candidate, 2014)

3.7.5. Novel Object Recognition Test (NORT)

The Novel object recognition test (NORT) is a commonly used behavioural assay for the investigation of various aspects of learning and memory in mice (Lueptow, 2017). Ennaceur and Delacour (1988) studied for the first time the novel object and novel location recognition tests(Antunes and Biala, 2012).

The NORT is fairly simple and is relatively low-stress, and is appropriate for the detection of neuropsychological changes following pharmacological, biological, or genetic manipulations and

can be completed over 3 days: habituation day, training day, and testing day. Duringthe Habituation day or orientation day, the mice were allowed to explore the empty arena for 2 minutes. On the Training day or exploration day, the mice were allowed to explore 2 identical objects placed at the same length for 10 minutes while on the Test day or experimental day, one of the two objects was replaced with a novel object and mice were allowed to explored for 5 minutes and time taken for novel and familiar object exploration were recorded (Huang *et al*., 2014).The apparatus was cleaned with alcohol between the test.



Plate VIII NORT apparatus(Snapped by the Candidate, 2016)

3.7.6 Beam walking Assay Test

The balance beam is a test of motor coordination and balance and can be used to detect subtle deficits in motor skills. This test is adapted from Tsenter *et al.*(2008).

Each mouse was placed on a brightly lit platform and was first allowed to transverse a beam 3cm width and 0.5cm in diameter to reach an enclosed safety box at the end of the beam during the training phase. During the test phase performance on the beam was quantified by measuring the time it takes for the mouse to traverse the narrow beam 2cm widthand 0.5 cm in diameter to an enclosed safety box, the number of foot slips were also recorded for a maximum of 60 seconds (1 Minute)(Stanley *et al*., 2012).

This task is particularly useful for detecting subtle deficits in motor skills and balance, Brain injury, genetic manipulations, and pharmacological treatments alterations of motor skills, the goal of this test was for the mouse to stay upright and walk across an elevated narrow beam to a safe platform (Loung *etal*.,2011).



Plate IXBeam walking Assay Test Apparatus (Snapped by the Candidate, 2016)

3.9 Statistical Analysis

All values were evaluated using SPSS version 22.0 software.Data were represented as means ± SEM and analyzed using One-way (treatment) or two-way (treatment × time) Analysis of Variance (ANOVA) with repeated measurement on the time factor where appropriate, followed by the Bonferroni *post hoc* test where treatment × time interaction were significant, where$p<0.05$ were considered significant when compared with control group.

CHAPTER FOUR

4.0 Results

4.01 The Phytochemical Screening of the *Ziziphus mauritiana*Leaf

The phytochemical analysis of *Ziziphus mauritiana* leaf revealed the presence of the variousmedicinally active phytoconstituents such as alkaloids,carbohydrates, cardiac glycosides, flavanoids, saponins, steroid and triterpenes and tanins. However, anthraquinones were absent (Table 1).

Table 4.1 PhytochemicalConstituents of the Analysis of Methanol Leaf Extract of *Ziziphus mauritiana*

Constituents	**Test**	**Inferences**
Carbohydrates	Molisch test	+
Anthraquinones	Bontrager's test	–
Glucosides	Fehling test	+
Cardiac glycosides	Kelle Killiani test	+
Saponins	Frothing test	+
Steroid and interpenes	Lieberman Burchard test	+
Tannins	Ferric Chloride test	+
Flavonoids	Shinoda test	+
Alkaloids	Dragendorff test	+

Keys

\+ _____ Presence

_ _____ Absence

4.0.2. Acute Toxicity Study of Methanol Leaf Extract of *Ziziphus mauritiana*

The acute toxicity study showed no toxicity, no death was observed .Therefore LD_{50} of Methanol leaf extract of *Ziziphus mauritiana* was estimated to be above 5000mg/Kg body weight.

4.0.3 Assesment of the Methanol Leaf Extract of *Ziziphus mauritiana* on Motor Coordination using Beam Walking Assay Test in Mice

Interestingly, methanol leaf extract of *Ziziphus mauritiana* treated group at the dose of 100mg/kg significant increase the time taken to complete the task when compared with the control groups,[F (5, 29)= 0.969, $p<0.001$](Appendix Table1).However, the extract treated group showed an increased in number of foot slips but it was not statistically significant difference when compared with the control [F (5, 29)=0.969,$p>0.05$].

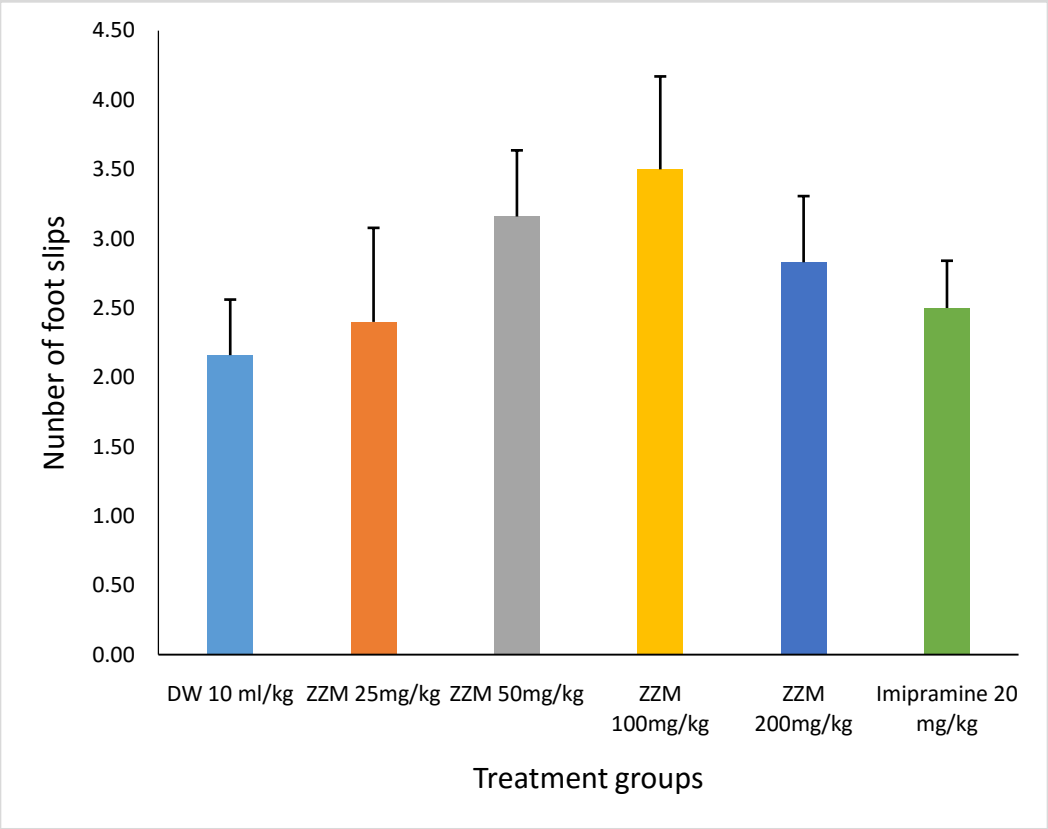


Figure 4.1Effect of theMethanol Leaf Extract of *Ziziphus mauritiana* on Motor Coordination in Mice

Animals were acutely treated with Methanol Leaf Extract of *Ziziphus mauritiana* (25, 50,100,200mg/kg *po*), Distilled water (10ml/kg),or Imipramine(20mg/kg *po*). Each column representsthe mean±SEM of 6 animals.Data was analyzed using one way ANOVA. *P>0.05* not significantly different with distilled water treated animal. ZZM=Methanol Leaf Extract of *Ziziphus mauritiana*, DW= Distilled water.

4.1.0Antidepressant Study of the Methanol Leaf Extract of *Ziziphus mauritiana* on Acute Model of Depression in Mice

4.1.1 Effect of the MethanolLeaf Extract of *Ziziphus mauritiana* on Duration of Immobility on Tail Suspension Test (TST) in Mice.

The extract treated groupsat 25mg/kg and 200mg/kgshowed statistical significantly decrease in the duration of immobility when compared with the control[$F\ (5,\ 30) = 29.401, p < 0.001$]. However, there was also decrease in duration of immobility (Behavioural despair) inthe Imipramine 20mg/kg treated group which is statistically significant.

Table 4.2. Effect of the Methanol Leaf Extract of *Ziziphus mauritiana* on Immobility Time (TST)in Mice

Treatment Group	Immobility time (sec)
DW 10 ml/kg	309.50 ±10.93
ZZM 25 mg/kg	183.50 ±17.64*
ZZM 50 mg/kg	275.83±7.15
ZZM 100 mg/kg	270.12±6.97
ZZM 200 mg/kg	198.00 ±8.74*
Imipramine 20 mg/kg	137.00±16.90*

Animals were acutely treated with MethanolLeaf Extract of *Ziziphus mauritiana* (25, 50,100, 200 mg/kg *po*), Distilled water (10ml/kg),or Imipramine (20mg/kg *po*). The table represent the mean ±SEM of 6 animals. Data was analyzed using one way ANOVA followed by Bonferroni post hoc test,*$p \leq 0.000$ significantly different from Distilled water treated animals. ZZM=Methanol Leaf Extract of *Ziziphus mauritiana,* DW= Distilled water

4.1.2 Effect of the Methanol Leaf Extract of*Ziziphus mauritiana* on Behavioural Despair Force Swimming Test (FST) in Mice

The duration of immobility in Forced Swim test (FST), between the extract treated group and control was notstatistically significant [F (5, 30) =7.072, *p>0.05]*. However, there was statistical significance difference at 100mg/kg of Methanol Leaf Extract of *Ziziphus mauritiana* treated animal which showed increase in immobility time while decrease in duration of immobility was seen in Imipramine 20mg/kg and control (10ml/kg).

Table 4.3 Effect of the MethanolLeaf Extract of *Ziziphus Mauritiana* on Duration of Immobility of Mice in FST (Forced Swim Test)

Treatment Group	Immobility time (Sec)
DW 10 ml/kg	117.00±7.84
ZZM 25 mg/kg	152.33±16.83
ZZM 50 mg/kg	160.33±14.93
ZZM 100 mg/kg	165.00±10.30*
ZZM 200 mg/kg	158.33±9.28
Imipramine 20 mg/kg	85.50±9.61*

Animals were acutely treated with MethanolLeaf Extract of *Ziziphus mauritiana* (25, 50,100, 200 mg/kg *po*), Distilled water (10ml/kg),or Imipramine (20mg/kg *po*). The Table represent the mean ±SEM of 6 animals. Data was analyzed using one way ANOVA.*$p \leq 0.05$significantly different from Distilled water treated animals. ZZM=MethanolLeaf Extract of *Ziziphus mauritiana*, DW= Distilled water

4.1.3 Effect of theMethanol Leaf Extract of*Ziziphus Mauritiana*on Locomotion of mice in Open Field Test (OFT) by Mice

There were no statistically significant difference in the numbers of line crossing among the animals treated with the methanol leaf extract of *Ziziphus mauritiana*, distilled waterand Imipramine[$F(5,30)=2.407, p>0.05$].

4.4 Effect of theMethanol Leaf Extract of*Ziziphus mauritiana* on Gross Locomotion in Open Field Test (OFT) by Mice.

Treatment Group	Frequency of Line crossing
DW 10 ml/kg	109.17±11.91
ZZM 25 mg/kg	84.33±14.78
ZZM 50 mg/kg	111.33±12.91
ZZM 100 mg/kg	78.33±11.06
ZZM 200 mg/kg	113.00±8.09
Imipramine 20 mg/kg	76.50±10.04

Animals were acutely treated with Methanol Leaf Extract Methanol of *Ziziphus mauritiana* (25, 50,100, 200 mg/kg *po*), Distilled water (10ml/kg),or Imipramine (20mg/kg *po*).The Table represent the mean ±SEM of 6 animals. Data was analyzed using one way ANOVA. $p > 0.05$ not significantly different from Distilled water treated animals. ZZM=Methanol Leaf Extract of *Ziziphus mauritiana,* DW= Distilled water

4.1.4Effect of theMethanol Leaf Extract of*Ziziphus Mauritiana* on Locomotion of Mice on Rearing Behaviour in Open Field Test in Mice

There were no statistically significant difference in rearing activity among the animals treated with the distilled water,methanol leaf extract of *Ziziphus mauritiana*, and Imipramine [$F(5,30)=0.849, p>0.05$].

4.5Effect of theMethanol Leaf Extract of*Ziziphus Mauritiana* on Locomotion of Mice on Rearing Behaviour in Open Field Test in Mice

Treatment Group	Frequency of Rearing
DW 10 ml/kg	3.17±1.30
ZZM 25 mg/kg	2.33±0.76
ZZM 50 mg/kg	3.00±0.63
ZZM 100 mg/kg	2.83±0.70
ZZM 200 mg/kg	2.12±0.75
Imipramine 20 mg/kg	1.17±0.31

Animals were acutely treated with Methanol Leaf Extract of *Ziziphus mauritiana* (25, 50,100, 200 mg/kg *po*), Distilled water (10ml/kg),or Imipramine (20mg/kg *po*).The Table represents the mean ±SEM of 6 animals. Data was analyzed using one way ANOVA. *P> 0.05* not significantly different from Distilled water treated animals. ZZM=MethanolLeaf Extract of *Ziziphus mauritiana*, DW= Distilled water

4.1.5 Effect of theMethanol Leaf Extract of *Ziziphus mauritiana*on Novel Object Recognition Memory (NORT) in Mice

There were no statistically significant difference in novel object exploration and familial object exploration among the animals treated with the distilled water,Methanol leaf extract of *Ziziphus mauritiana,* and Imipramine F(5,30)=0.536, $p>0.05$],F(5,30)=2.572,$p>0.05$] respectively (Appendices Table 2 and 3). However,There was also no statistically significant difference in percentage preference in novel object exploration among the animals treated with the distilled water,Methanol leaf extract of *Ziziphus mauritiana,* and Imipramine [F(5,30)=1.786,$p>0.05$].

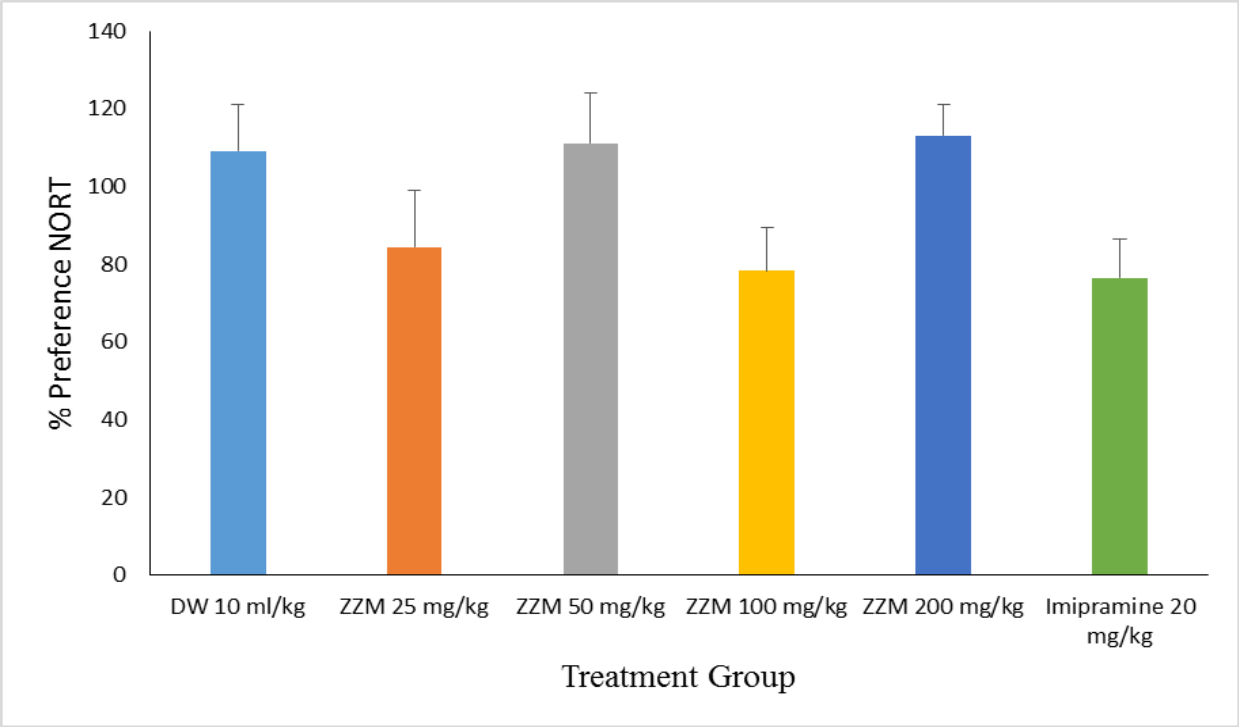


Figure 4.2 Effect of the Methanolleaf extract of *Ziziphus Mauritiana*on percentage preference in Mice

Animals were acutely treated with Methanol Leaf Extract of *Ziziphus mauritiana* (25, 50,100, 200 mg/kg *po*), Distilled water (10ml/kg),or Imipramine (20mg/kg *po*). Each column represents the mean ±SEM of 6 animals. Data was analyzed using one way ANOVA. *p> 0.05* not significantly different from Distilled water treated animals. ZZM=Methanol Leaf Extract of *Ziziphus mauritiana*, DW= Distilled water

4.2 Effect of MethanolLeaf Extract of *Ziziphus Mauritiana*on BCG (*Bacillus Calmette-Guerin*) Induced Depression Model in Mice

The effect of the Methanolleaf extract of *Ziziphus Mauritiana* on sickness behavior induced by BCG, assessed 3 classical sickness, namely body weight loss, febrile response and locomotory impairment.

4.2.1 Effect of theMethanol Leaf Extract of *Ziziphus Mauritiana* on Body Weight in Mice following BCG treatment

There were statistical significant increase in body weight between the methanol leaf extract of *Ziziphus Mauritiana* treated group and imipramine group from 1sthour -21st days post inoculation [(F(6,30) = 2.507, p < .0044)],pre and post BCG inoculation treatment were also significant among all the treatment groups [F(5.616) = 106.67, p < .001].DW+BCG group significantly decrease body weight 0 hour- 21st days post inoculation whencompared to the other groups [F (3.56) = 17.08, p < .000) p<0.001).

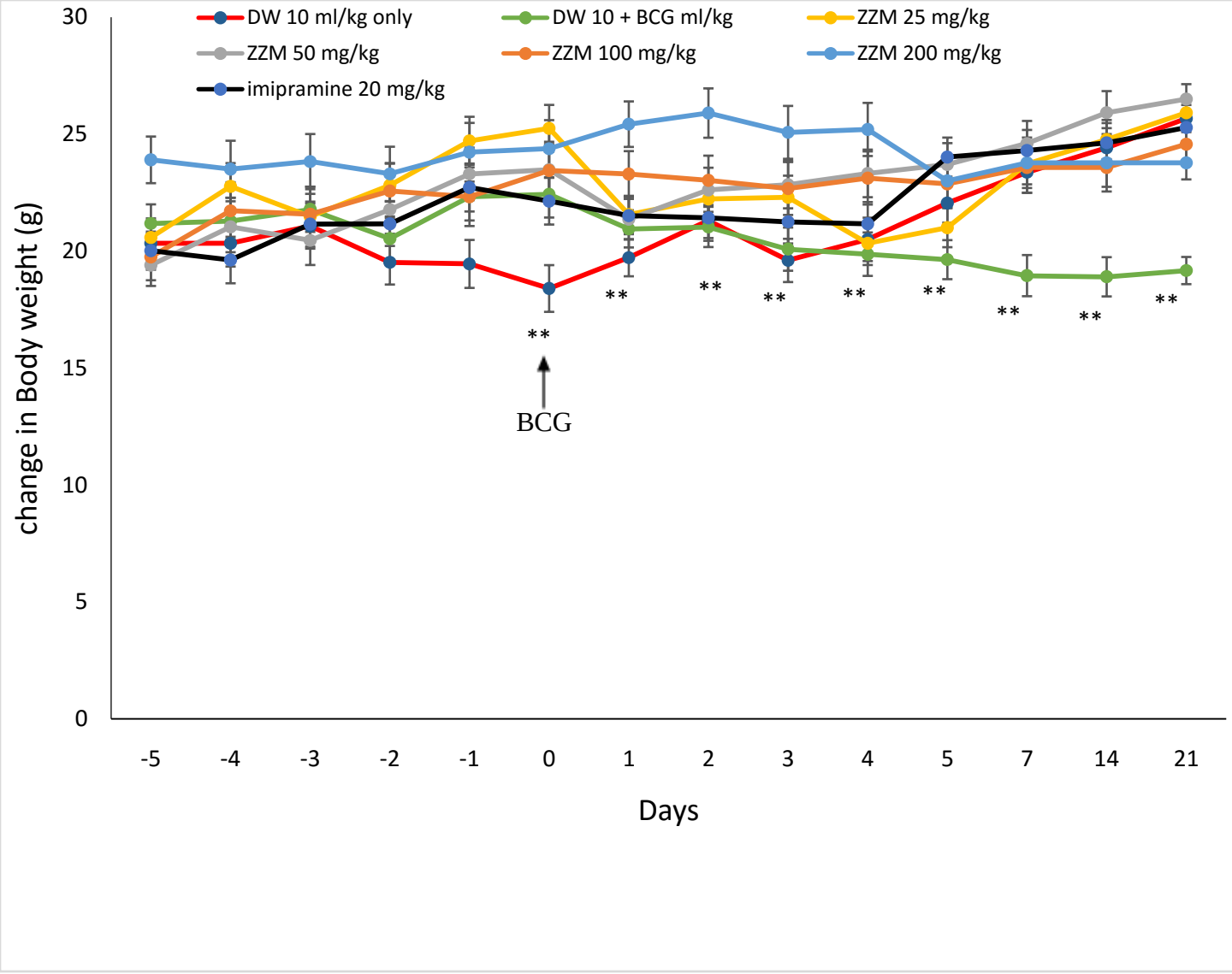


Figure 4. 3. Effect of the Methanol Leaf Extract of *Ziziphus mauritiana* on Body Weight in Mice following treatment with BCG

Animals were inoculated withBCG (0.2mg/kg *ip*) and were chronically treated with Methanol Leaf Extract of *Ziziphus mauritiana* (25, 50,100, 200 mg/kg *po*), Distilled water (10ml/kg),or Imipramine (20mg/kg *po*). Data were expressed as mean ±SEM of 6 animals and analyzed using two way ANOVA followed by Bonferroni post hoc, *$p \leq 0.001$significantly different from DW+BCG treated animal, ZZM= Methanol Leaf Extract of *Ziziphus mauritiana*, DW= Distilledwater, BCG = *Bacillus Calmette-Guerin.*

4.2.2. Effect of the Methanol Leaf Extract of *Ziziphus Mauritiana* on Body Temperature (rectal) in Mice

There was statistical significant decrease in body temperature between the methanol leaf extract of *Ziziphus Mauritiana* treated group and imipramine group from 4th hour -21st days post inoculation, (F (35.34,200.243)=42.10, p<0.000)], pre and post BCG inoculation treatment also showed a significant changes in body temperature among all the treatment groups overtime [(F(5.889,200.243)=12.31, p < .000)]. DW+BCG group significantly increased body temperature0 hour-21st days post inoculation when compared with the other groups [F (35.34, 200.243)=42.10, p<0.000)].

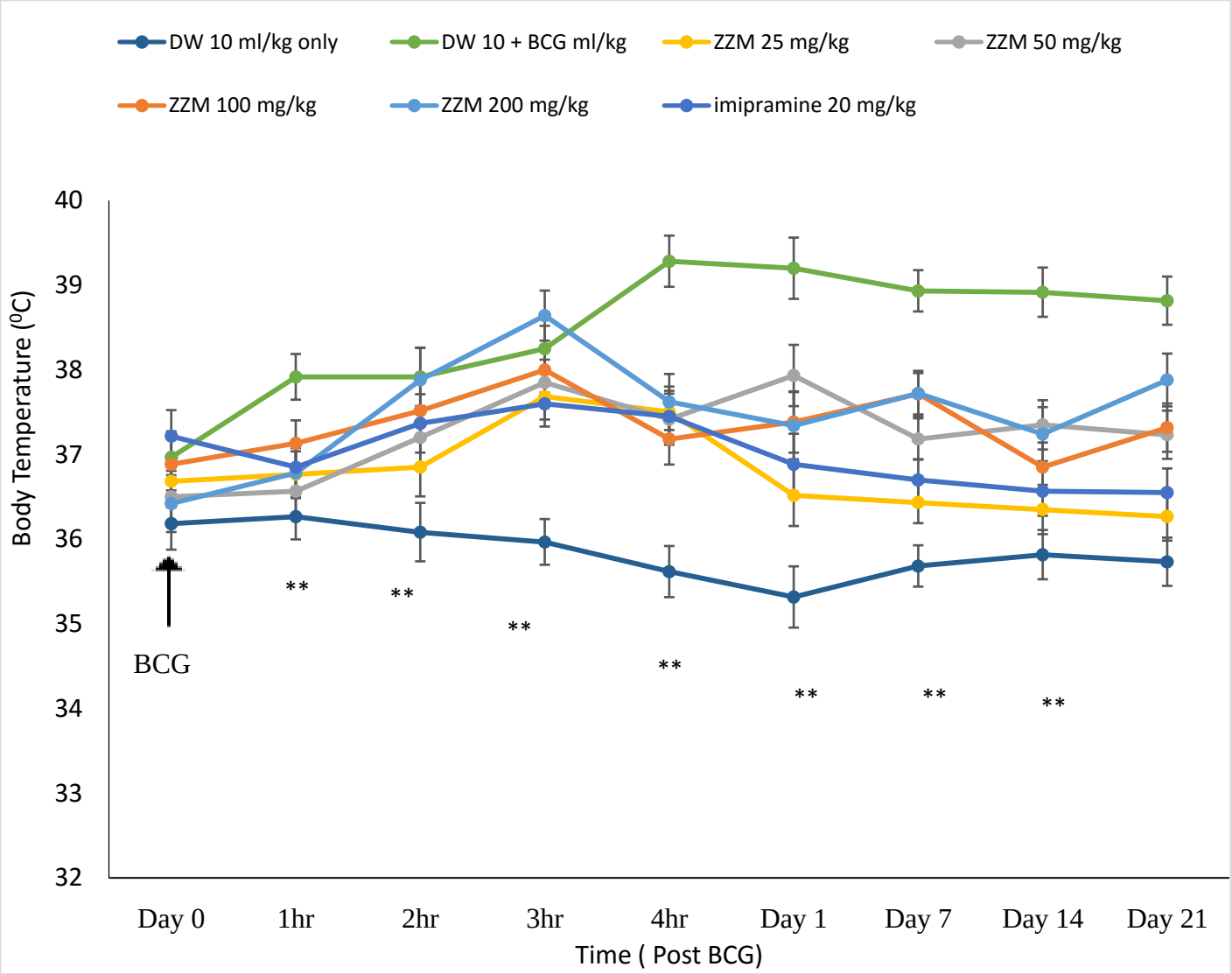


Figure 4.4 Effect of the Methanol Leaf Extract of *Ziziphus mauritiana*on Body Temperature

Animals were inoculated withBCG (0.2mg/kg *ip*) and were chronically treated with Methanol Leaf Extract of *Ziziphus mauritiana* (25, 50,100, 200 mg/kg *po*), Distilled water (10ml/kg),or Imipramine (20mg/kg *po*). Data were expressed as mean ±SEM of 6 animals and analyzed using two way ANOVA followed by Bonferroni post hoc, *$p \leq 0.001$significantly different from DW+BCG treated animal, ZZM= Methanol Leaf Extract of *Ziziphus mauritiana*, DW= Distilledwater, BCG = *Bacillus Calmette-Guerin.*

4.2.3 Effect of the Methanol Leaf Extract of *Ziziphus mauritiana* on Locomotion of mice following inoculation with BCG

There was statistically significantincrease in number of line crossing among the methanol leaf extract of *Ziziphus mauritiana*treated group when compared with DW+BCG at 8^{th}, 24^{th} and 48^{th} hours post inoculation [$F(3,69)=7.591, p<0.000$)], the extract of treated group also showedsignificantincrease in number of line crossing when compared with DW only group at 4^{th} and 48^{th} post inoculation [$F(6,23)=5.354, p<0.00$)]. However,there was statistically significantincrease in number of line crossing among the Imipraminetreated group when compared with DW+BCG or DW only group at 8^{th}, 24^{th} and 48^{th} hours post inoculation [$F(3,69)=7.591, p<0.000$)],

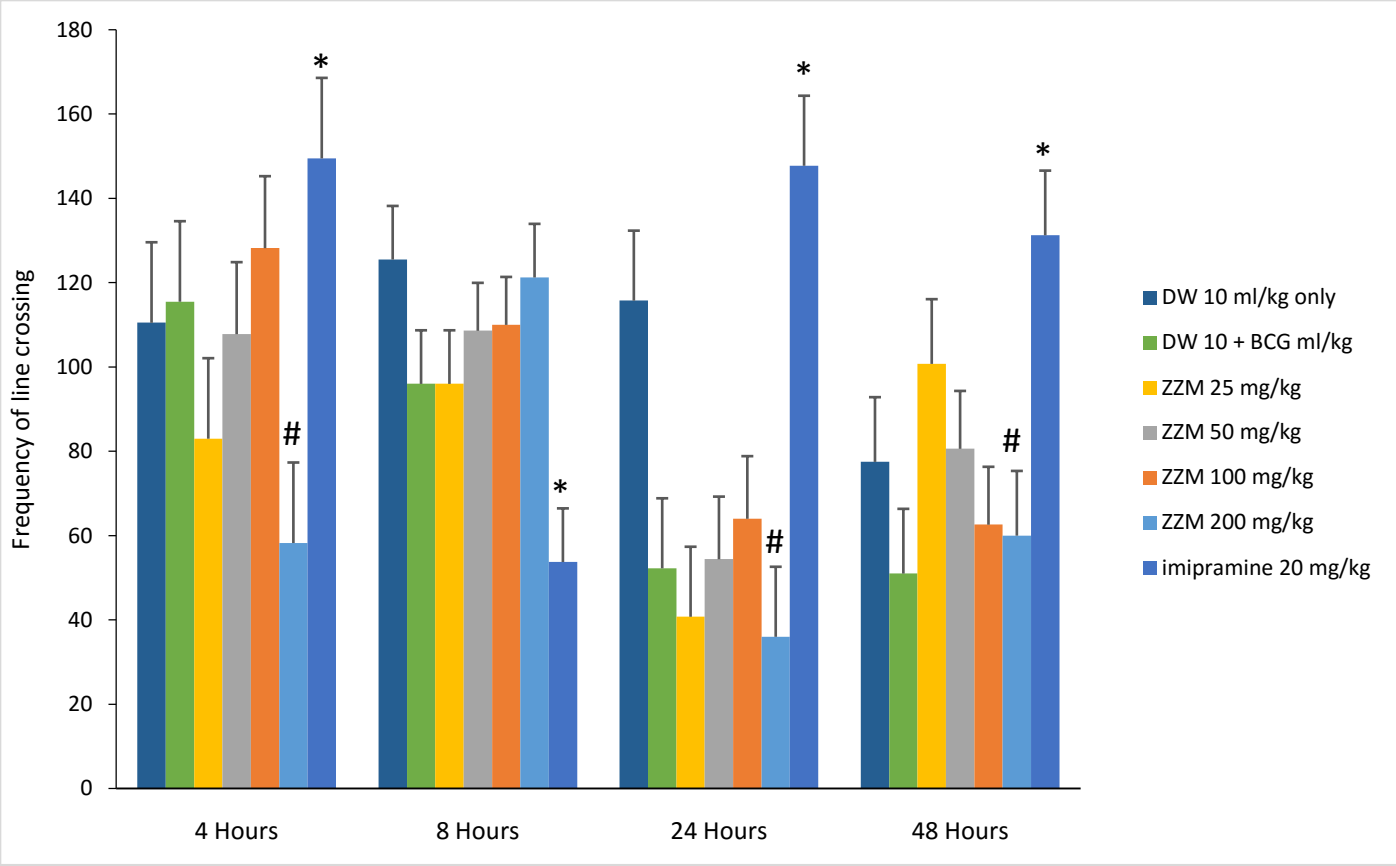


Figure 4.5 Effect of the Methanol Leaf Extract of *Ziziphus mauritiana*on Locomotion (Line crossed) of Mice following BCG inoculation

Animals were inoculated withBCG (0.2mg/kg *ip*) and were chronically treated with Methanol Leaf Extract of *Ziziphus mauritiana* (25, 50,100, 200 mg/kg *po*), Distilled water (10ml/kg),or Imipramine (20mg/kg *po*). Each column represents the mean ±SEM of 6 animals. Data was analyzed using two way ANOVA followed by Bonferroni post hoc, *$p \leq 0.00$significantly different from DW+BCG treated animal,#$p \leq 0.00$ Significant when compared with DW only group.

4.2.4. Effect of the Methanol leaf extract of *Ziziphus mauritiana* on Sucrose Preference Test (Anhedonia) in Mice.

There was statistically significant increase in sucrose consumption in the methanol leaf extract of *Ziziphus mauritiana*treated group and Imipramine groupat 1^{st} ,7^{th} , and 21^{st} day post inoculation when compared with the DW+BCG orDW only group [$F(6,21)=11.756$,$p<0.000$] .However,there was statistically significant drastic decrease in sucrose consumption in the DW+BCG group at 1^{st},7^{th}, and 21^{st} day post inoculation [$F(6, 21) =11.756$, $p<0.000$].

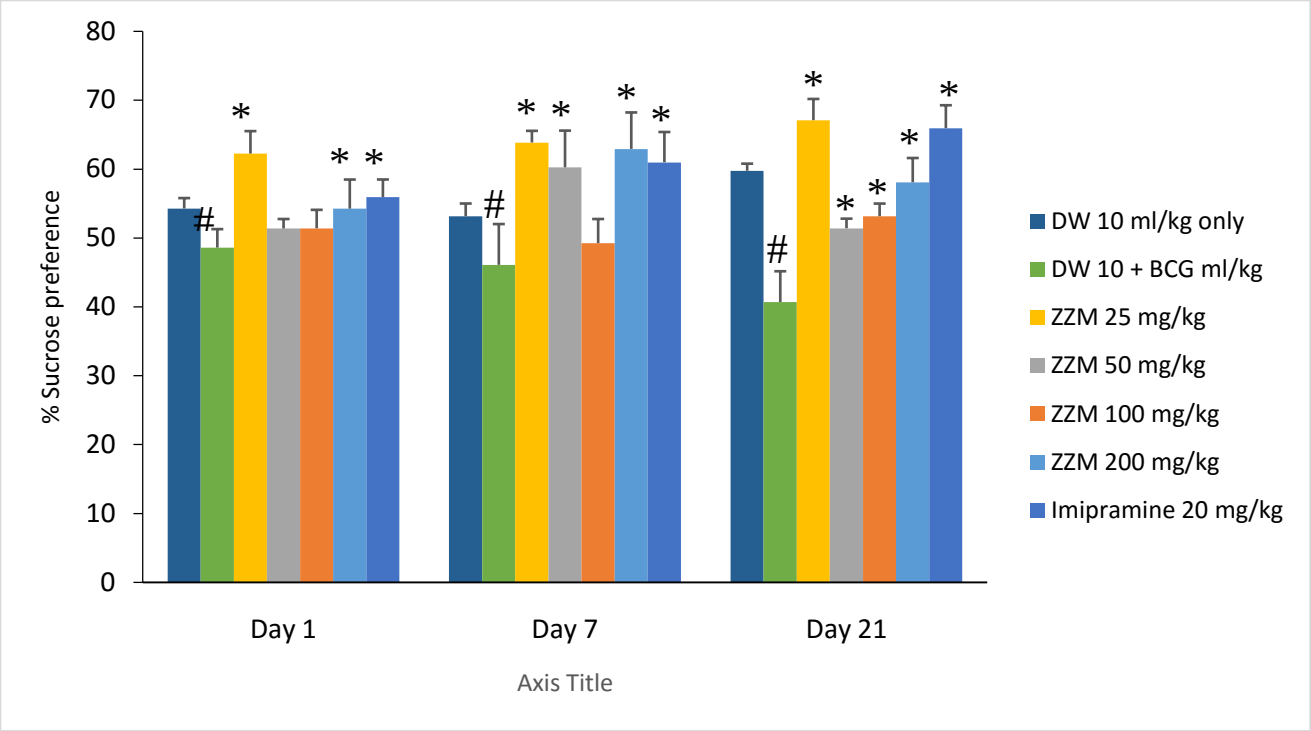


Figure 4.6 Effect of the Methanol leaf extract of *Ziziphus Mauritiana* on Percentage Sucrose Preference in Mice

Animals were inoculated with BCG (0.2mg/kg *ip*) and were chronically treated with Methanol Leaf Extract of *Ziziphus mauritiana* (25, 50,100, 200 mg/kg *po*), Distilled water (10ml/kg), or Imipramine (20mg/kg *po*). Each column represents the Mean ±SEM of 6 animals. Data was analyzed using two way ANOVA followed by Bonferroni post hoc, *$p \leq 0.001$ significantly different from DW+BCG treated animal, #$p \leq 0.001$Significant when compared with DW only group, ZZM= Methanol Leaf Extract of *Ziziphus mauritiana,* DW= Distilled water, BCG = *Bacillus Calmette-Guerin.*

4.2.5 Effect of the MethanolExtract of *Ziziphus mauritiana*on Duration of Immobility of mice inTail Suspension Test (TST)

Therewasstatistically significant decrease in the duration of immobility in the Methanol leaf extract of *Ziziphus mauritiana* treated animals at 21st day post inoculation when compared with the DW+BCG [F(8.370,40.453)=2.965,p<0.010)]. However, there was alsostatistically significant increase in duration of immobility in the DW+BCGtreated animals at 14th and 21stday post inoculation when compared with the extract treated group, DW only group and Imipramine group [F(6,29)=4.122,*p<0.004*)].

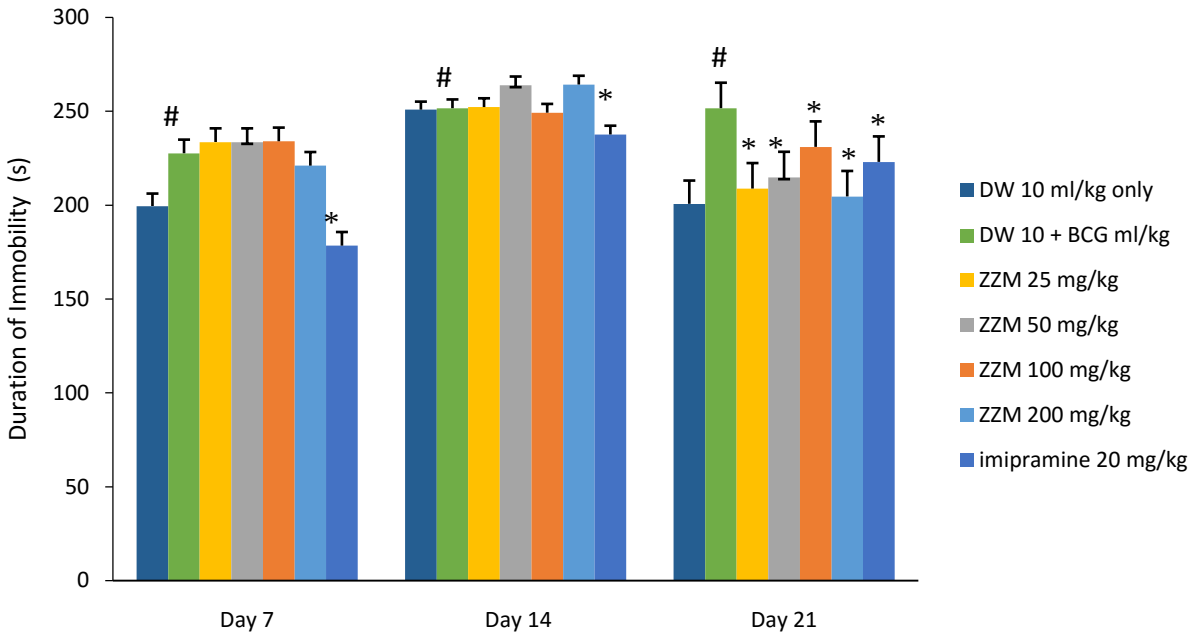


Figure: 4.7 Effect of the Methanolleaf Extract of *Ziziphus Mauritiana* on Duration of Immobilityof mice in Tail Suspension Test (TST)

Animals were inoculated with BCG (0.2mg/kg) and were chronically treated with the Methanol leaf extract of *Ziziphus Mauritiana* (25, 50, 100, and 200mg/kg*po*), Distilledwater (10ml/kg), orImipramine (20mg/kg*po*).Each column represents the Mean SEM± of 6 animals.Data was analyzed using two way ANOVA followed by Bonferoni post hoc test,*$p<0.000$ is significant when compared with DW+BCG, # $p<0.004$ is significant when compared with DW, ZZM=The Methanol leaf extract of *Ziziphus mauritiana*,DW= Distilled water, BCG= *Bacillus Calmette-Guerin*

CHAPTER FIVE

5.0 Discussion

Depression is a neuropsychiatric disorder affecting millions of people each year. Modern treatments are expensive, complex and inaccessible for many African population and medicinal plants have been an alternative sources of important therapeutic agent in mankind.Therefore, this study was designed to study the antidepressant activity of the of methanol leaf extract of the *Ziziphus mauritiana* in Swiss albino mice in acute study using Tail Suspension Test, Forced Swimming Test, Open Field Test, and Novel Object Recognition Test. Similarly, chronic study was also conducted on BCG induce depression model using Open Field Test, Tail Suspension Test, and Sucrose Preference Test in Swiss albino mice.

This study demonstrated that exposure of the mice pretreated with the Methanol leaf extract of *Ziziphus mauritiana* to tail suspension test (TST) induced a marked decrease in duration of immobility compared to control in a U shape dose response mode (25,200,100, and 50mg/kg),the extract alleviated the depressive like behavior induced by TST and this may suggest that the extract possessed antidepressant activity and this is in agreement with the finding of Liu *et al* (2012) who reported the antidepressant like effect of the ethanolic extract of *Ziziphus* specie in rat. The reduction of duration of immobility in the test may be due to activation of catecholaminergic mechanism in the brain (Afria *et al*., 2015).Contrarily, increased in duration of immobility in Methanol leaf extract of *Ziziphus mauritiana* treated animalswas observed in Forced swim test. Several studies indicated that immobility, swimming and climbing behaviours are displayed by different classes of antidepressant agents. The activity pattern showed by imipramine of increased climbing without swimming modifications is the pattern showed by agents that act via the adrenergic 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). Therefore, increase in duration of immobility observed in Forced swim test was probably due to aversion to water.However,the ability of the methanol leaf extract of *Ziziphus mauritiana* to reduce duration immobility in the TST has further ascertain its antidepressant activity and probably the utilization of adrenergic pathways (Shehu *et al*., 2017)

This work established that the methanol leaf extract of *Ziziphus mauritiana* was observed not to be associated with any motor coordination effects, due to insignificant change in number offoot slips of mice pretreated with the extract in comparison with control in the beam walking assay. This indicates that the extract never inhibit central nervous system (CNS) within the tested dose range. Additionally,Open field test was conducted to further eliminated the probability of false positive results in FST and TST as it was observed that there was insignificant change in locomotion activity(number of line crossing) in Methanol leaf extract of *Ziziphus mauritiana* treated groups in comparison with the control and this suggested that the extract does not possessed psychostimulant effect and this ensured that the increased active behaviour in the TST was not as a result of CNS stimulatory effect and confirmed the specificity of antidepressant like effect of the extract.In the NORT, the Methanol leaf extract of *Ziziphus mauritiana* treated animals showed insignificant increase in novel object exploration, familial object exploration, andpercentage preference for novel object when compared with the control and this suggested that the extract neither impairednor improvedcognitive function (learning and memory capacities)following acute administration at the tested doses in mice.The work of Sadiq *et al*.(2009) stated that *Ziziphus mauritiana*seed extract has improve spatial recognition memory on

rats as measuredby the Y –Maze and Une *et al*.(2012) also stated that the n-butanolic fraction of methanol extract of leaves of *Ziziphus mauritiana* antagonized the amnesic effect of scopolamine, improvement in learning, memory andcognition on the NORT in mice.

In chronic study,themethanolleaf extract of *Ziziphus mauritiana attenuated* BCG induced depressive like behaviour in mice as evidenced in the decrease in duration of immobility of BCG-pretreated mice after treating with the various doses of the extract. *Bacillus Calmette-Guerin*(BCG), inoculation was reported to induced an acute episode of sickness (approximately 5 days) that was followed by development of delayed depressive-like behaviors lasting over several weeks as a result of activation of IDO by proinflammatory cytokines that alters serotoninergic and glutamatergic neurotransmission since tryptophan is the limiting factor for the synthesis of serotonin, decreased circulating tryptophan concentration have the potential to negatively impact serotoninergic neurotransmission(O'Connor *et al*., 2009).Therefore, chronic administration of the extract at 21st day attenuate the depressive like effect of the BCG by significantly decreasing the duration of immobility in mice .Furthermore, the delayed antidepressant effect of the extract seen here was as a result of prolong depressive-like behavioural effects of BCG that were easier to evidence on the long-term when mice were tested only once than when they were submitted to repeated behavioral testing, or may be due to the delayed establishment of this long-term depressive-like behavior by BCG probably reflects the time required for the cytokine-induced neurobiochemical changes underlying depressive-like symptoms to fully develop (Moreau *et al.*, 2008).

Interestingly, mice pretreated with BCG reversed the altered locomotor activity induced by BCG in mice without further stimulant action.The extract increased general locomotor activity at 8th hour, 24th hour and 48th hour post inoculation evidenced by increase in frequency of line

crossing, when compared with the DW+ BCG group. This result supported the fact that antidepressant action of methanol leaf extract of *Ziziphus mauritiana* was not associated with stimulating locomotor activity. On the other hand imipramine at 8th hourdid not reverse the altered locomotor activity induced by BCG as it was reported by Duda *etal*.(2016) that chronic administration of imipramine at a doses of 10mg/kg and above inhibit locomotor activity in rodents . Therefore chronic administration of the extract at 48th hour was able to ameliorate inflammation-induced depressive-like behaviours by BCG which may be precipitated due to increase IDO (indoleamine 2,3-dioxygenase) activity that metabolizes the tryptophan, the precursor of 5-HT(serotonin) to neurodegenerative quinolinate and neuroprotective kynurenate (Myint and Kim, 2003),Increase in IDO activity have the potential to negatively impact serotonin and serotoninergic neurotransmission by decreasing bioavailability of tryptophan (Marcus, 2013),thus, increase degradation of tryptophan along the kynurenine pathway. This might indicate that the extract target IDO (indoleamine 2,3-dioxygenase) activity or it inhibits BCG induced depressive-like behaviour that is associated with blockade of brain proinflammatory cytokine and indoleamine 2,3-dioxygenase (IDO) up regulation and this may provide novel therapeutic treatment strategies in patients suffering from inflammation associated depression(O'Connor *et al*., 2009).The imipramine treated animal showed a decrease in number of line crossing at 8th hour but later improved at 24th hour and 48th hour.In addition, DW+BCG groups exhibited more depressive like behaviour evidenced by decreasing the frequency of line crossing even after 48th hours following inoculation,thismay be due toprolonged increase in circulating concentrations of tryptophan (TRP) that is accompanied by elevated concentrations of kynurenine (KYN),activated by TRP-catabolizing enzyme indoleamine 2,3-dioxygenase (IDO) (Maes, 2009).

This study also investigated the effect of methanol leaf extract of *Ziziphus mauritiana* on BCG induced depressive-like behaviour in the sucrose preference test, an animal test ofdepression which directly models a core symptom of depression, namely anhedonia, i.e. the lack of interest in pleasurable activities including consumption of palatable foods (Moreau *et al.*,2008). Interestingly, the methanol leaf extract of *Ziziphus mauritiana* treated groups showed increased sucrose intake at 24th hours (1st day), 7th day and 21st day post inoculation more than the DW only group, DW+BCG groups with pronounced biological efficacy at 25mg/kg and 200mg/kg. The 14th day sucrose intake was not measured in the experiment to avoid habituation in mice. The extract was able to ameliorate anhedonia, (improve mood) which is a core symptom of the depressive-like behaviour induced by chronic inflammation, and cure depressive symptoms (anhedonia) for a long period of 3(three) weeks post inoculation

The methanol leaf extract of*Ziziphus mauritiana*treated animals showed time dependent increase in body weight after BCG inoculation. Transient loss of body weight shortly after BCG inoculation in all the groups was observed,but the methanol leaf extract of *Ziziphus mauritiana* groups were able to increase body weight 1-7th day post inoculation in a dose nondependent manner. On the other hand DW+BCG groupsshowed decrease in body weight from inoculation hour up to 7th day post inoculation.The phytochemical analysis of the extract revealed the presence of carbohydrate, alkaloids and flavonoids, saponins which may be expected to be responsible for its bioactivity,the work of Guo *et al*. (2017) stated that *Ziziphus* plants contained carbohydrate, vitamins, fatty acid, amino acids and nucleosides which are valuable nutrients and are reportedly involved in the regulation and modulation of various physiological processes in the body, which all contribute to human health and it also exhibit antioxidant activity.

Abdulameer *et al.*(2017) stated that oral administration of hydroalcholic *Ziziphus mauritiana* leaves extract (AZL) can be used as alternatives growth performances in broiler chicken.

Furthermore, the methanol leaf extract of *Ziziphus mauritiana* exhibit antipyretic effect on BCG induced depressive behaviour model. It was observed that BCG induced a significant increase in core body temperature in DW+BCG group 1hour –21stday post-inoculation. The increase in body temperature occurred 3-4hour and remained at peak 4hour-21st day post inoculation, while decrease in body temperature was observed 1hour-21stday post inoculation in methanol leaf extract of *Ziziphus maur*itiana treated groups and imipramine group (standard drug).The extract was able to ameliorate the effect of acute sickness response(elevated febrile response) following infection or inflammation induced by BCG best at 25 mg/kg (Moreau *et al*., 2008). The work of AbdulRauf *et al*. (2016) stated the root extract of *Ziziphus nummularia* which is a *Ziziphus* specie exhibits remarkable antipyretic effects in yeast induced hyperthermia in mice. These findings explain the rationale of traditional medicinal use of *Ziziphus nummularia* for alleviating fever and also Aye *et al*. (2013) stated that the *Ziziphus jujube a Ziziphus specie* reduces the body temperature and sweat.

The exact mechanism underlying antidepressant activity of the methanol leaf extract of *Ziziphus mauritiana* was difficult to predict, since it behaved in a dose nondependent mode, throughout the studies,yet it could be due to decrease in activation of IDO pathway or by decreasing degradation of tryptophan along the kynurenine pathway or through its anti-inflammatory effect and the work of Abedini *et al. (*2015) established that the *Ziziphus Jujube* (Jujube) plant a *Ziziphus* specie has exhibited numerous medicinal and pharmacological properties including antioxidant and anti-inflammatory effects.

CHAPTER SIX

6.0 Conclusion and Recommendations

6.1 Conclusion

Based on the present experiment, the methanol leaf extract of *Ziziphus mauritiana* possesses antidepressant activity possibly mediated via its anti-inflammatory property probably via decreasing IDO (indoleamine 2,3-dioxygenase) activity, it also possessedantipyretic effect and improved body weight loss and attenuated anhedonia in BCG induced depressive like behaviour.

6.2 Recommendations

i. Future studies are required to further elucidate the relationship between the inflammation pathway and the antidepressant effect of the methanol leaf extract of *Ziziphus mauritiana*.

ii. Molecular study to confirm the exact mechanism of action of the methanol leaf extract of *Ziziphus mauritiana* on depression is essential.

iiiGas Chromatograph Mass Spectroscopy analysis to determine the possible phytochemicals that may be responsible for the antidepressant activity of the methanol leaf extract of *Ziziphus mauritiana*.

6.3 Contribution to knowledge

i This study show the acute toxicity of the methanol leaf extract of *Ziziphus mauritiana* is relatively safe for consumption (LD_{50}>5000 mg/kg).

ii This study show that the methanol leaf extract of *Ziziphus mauritiana* possesses antidepressant effect in TST {(25 mg/kg 183.50 ± 17.60,200mg/kg, (198.0 ± 8.74) f(6,29)=4.12, $p \leq 0.001$)}, and SPT {25 mg/kg, 64.40 ± 1.860 f(6,21)=11.76 $p < 0.001$)} in mice.

iii This study also show the involvement of inflammatory mechanism in the antidepressant activity of the methanol leaf extract of *Ziziphus mauritiana* in mice.

iv This study also show that the extract reduced body temperature at {25 mg/kg 36.78± 0.22,(f(6,34=13.40,$p<0.001$)} and improved body weight loss at {25 mg/kg 22.82 ± 0.88, f(6,30=2.51,p <0.004)} induced by BCG in mice.

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APPENDICES

Table 1effect of methanol leaf extract on *Ziziphus mauritiana* time taken to complete the task in Beam walking assay

Treatment Group	Time spent (sec)
ZZM 200 mg/kg	18.67±2.89
ZZM 100 mg/kg	28.67±2.80*
ZZM 50 mg/kg	21.83±2.91
ZZM 25 mg/kg	19.50±2.35
imipramine 20 mg/kg	15.17±1.82
DW 10 ml/kg	14.50±1.18

*The main difference is significance when compared with DW 10 ml/kg, $p<0.05$

ZZM*: Ziziphus mauritiana*

DW: Distilled water

Table 2 Effect of methanol leaf extract of *Ziziphus Mauritiana*on Novel object Exploration

Treatment Group	Frequency of exploration
ZZM 200 mg/kg	6.33±2.17
ZZM 100 mg/kg	5.5±1.41
ZZM 50 mg/kg	7.2±2.22
ZZM 25 mg/kg	4.5±2.03
imipramine 20 mg/kg	7.67±1.80
DW 10 ml/kg	8.33±2.14

Table 3 Effect of the methanol leaf extract of*ZiziphusMauritiana*on Familial object exploration

Treatment Group	Familiar Object Exploration
ZZM 200 mg/kg	5.5±2.1
ZZM 100 mg/kg	5.3±1.0
ZZM 50 mg/kg	5.4±1.7
ZZM 25 mg/kg	2.8±1.2*
imipramine 20 mg/kg	5.3±1.7
DW 10 ml/kg	11.6±2.8

*The main difference is significance when compared with DW 10 ml/kg, $p<0.05$

Table 4 Effect of aqueous leaf extract of *Ziziphus Mauritiana*on body weight

Time	Body weight (g)
Pre BCG- Day 1	20.710 ± 0.333*
Pre BCG- Day 2	21.466 ± 0.406*
Pre BCG- Day 3	21.620 ± 0.394*
Pre BCG- Day 4	21.670 ± 0.388*
Pre BCG- Day 5	22.724 ± 0.419*
Innoculation day (day 0)	22.789 ± 0.409
Post BCG day 1	21.976 ± 0.325
Post BCG day 2	22.504 ± 0.352
Post BCG day 3	21.975 ± 0.378
Post BCG day 4	21.930 ± 0.380
Post BCG day 5	22.325 ± 0.342
Post BCG day 7	23.186 ± 0.360
Post BCG day 14	23.712 ± 0.343
Post BCG day 21	24.412 ± 0.238*

***** The mean difference is statistically significant when compared with the inoculation day (day 0), $p<0.001$.

Similarly, there was a significant main effect of treatment group (6, 30) = 2.507,$p<0.004$.This result shows that there was significant difference in body weight between the treatment groups, irrespective of time.

Simple effect of treatment

Groups	Body weight(g)	Groups	Body weight(g)
Pre BCG day1		**Pre BCG day 2**	
ZZM 200mg/kg+BCG	23.90 ± 1.794	**ZZM 200mg/kg+BCG**	23.50 ± 2.573
ZZM 100mg/kg+BCG	19.75 ± 1.967	**ZZM 100mg/kg+BCG**	21.725± 2.423
ZZM 50mg/kg+BCG	19.40 ± 1.787	**ZZM 50mg/kg+BCG**	21.040 ±2.919
ZZM 25mg/kg+BCG	20.583 ± 1.486	**ZZM 25mg/kg+BCG**	22.767 ± 1.550
Imip 20mg/kg+BCG	20.017 ± 3.452	**Imip 20mg/kg+BCG**	19.617 ± 4.057
DW 10mL/kg+BCG	21.183 ± 1.5012	**DW 10mL/kg+BCG**	21.283 ± 1.151
DW 25mL/kg	20.33± 0.8287	**DW 25mL/kg**	20.333 ± 1.015

Groups	Body weight(g)	Groups	Body weight(g)
Pre BCG day3		**Pre BCG day 4**	
ZZM 200mg/kg+BCG	23.825 ± 2.389	**ZZM 200mg/kg+BCG**	23.300± 1.456
ZZM 100mg/kg+BCG	21.575 ± 2.576	**ZZM 100mg/kg+BCG**	22.575± 2.132
ZZM 50mg/kg+BCG	20.460±3.354	**ZZM 50mg/kg+BCG**	21.780± 3.143
ZZM 25mg/kg+BCG	21.483±0.857	**ZZM 25mg/kg+BCG**	22.817± 0.995
Imip 20mg/kg+BCG	21.150± 3.693	**Imip 20mg/kg+BCG**	21.167± 3.953
DW 10mL/kg+BCG	21.784± 1.245	**DW 10mL/kg+BCG**	20.533± 1.245
DW 25mL/kg	21.535± 2.335	**DW 25mL/kg**	19.517± 1.521

Groups	Body weight(g)	Groups	Body weight(g)
Pre BCG day5		**Innoculation (day 0)**	
ZZM 200mg/kg+BCG	24.225± 1.498	**ZZM 200mg/kg+BCG**	24.375±2.136
ZZM 100mg/kg+BCG	22.325±3.678	**ZZM 100mg/kg+BCG**	23.450±2.558
ZZM 50mg/kg+BCG	23.30±3.289	**ZZM 50mg/kg+BCG**	23.480±3.820
ZZM 25mg/kg+BCG	24.717±0.952	**ZZM 25mg/kg+BCG**	25.250±0.836
Imip 20mg/kg+BCG	22.717±3.965	**Imip 20mg/kg+BCG**	22.133±3.593
DW 10mL/kg+BCG	22.33±0.911	**DW 10mL/kg+BCG**	22.433±1.553

DW 25mL/kg	19.450±1.492	**DW 25mL/kg**	18.400± 0.522
Groups	**Body weight(g)**	**Groups**	**Body weight(g)**
Post BCG (day1)		**Post BCG day 2**	
ZZM 200mg/kg+BCG	25.425± 1.387	**ZZM 200mg/kg+BCG**	25.900±1.233
ZZM 100mg/kg+BCG	23.300±1.826	**ZZM 100mg/kg+BCG**	23.025±1.89
ZZM 50mg/kg+BCG	21.360±2.865	**ZZM 50mg/kg+BCG**	22.62±3.775
ZZM 25mg/kg+BCG	21.567±1.651	**ZZM 25mg/kg+BCG**	22.333±1.916
Imip 20mg/kg+BCG	21.517±2.589	**Imip 20mg/kg+BCG**	21.417±1.993
DW 10mL/kg+BCG	20.955±0.985	**DW 10mL/kg+BCG**	21.033±1.253
DW 25mL/kg	19.717±1.617	**DW 25mL/kg**	21.300± 1.736

Groups	**Body weight(g)**	**Groups**	**Body weight(g)**
Post BCG day3		**Post BCG day 4**	
ZZM 200mg/kg+BCG	25.075± 1.220	**ZZM 200mg/kg+BCG**	25.200±1.030
ZZM 100mg/kg+BCG	22.675± 2.089	**ZZM 100mg/kg+BCG**	23.125±2.870
ZZM 50mg/kg+BCG	22.840±4.048	**ZZM 50mg/kg+BCG**	23.320±3.696
ZZM 25mg/kg+BCG	22.300±1.575	**ZZM 25mg/kg+BCG**	20.333±2.251
Imip 20mg/kg+BCG	21.250±2.139	**Imip 20mg/kg+BCG**	21.167±1.941
DW 10mL/kg+BCG	20.083±1.065	**DW 10mL/kg+BCG**	19.867±1.017
DW 25mL/kg	19.600±2.428	**DW 25mL/kg**	20.500± 2.151

Groups	**Body weight(g)**	**Groups**	**Body weight(g)**
Post BCG day5		**Post BCG day 7**	
ZZM 200mg/kg+BCG	23.000± 1.667	**ZZM 200mg/kg+BCG**	23.775±2.092
ZZM 100mg/kg+BCG	22.875±2.039	**ZZM 100mg/kg+BCG**	23.575±2.802
ZZM 50mg/kg+BCG	23.700±3.584	**ZZM 50mg/kg+BCG**	24.600±3.478
ZZM 25mg/kg+BCG	21.00±1.923	**ZZM 25mg/kg+BCG**	23.733±2.378
Imip 20mg/kg+BCG	24.017±1.418	**Imip 20mg/kg+BCG**	24.300±1.423
DW 10mL/kg+BCG	19.633±0.918	**DW 10mL/kg+BCG**	18.950±0.709
DW 25mL/kg	22.050±2.029	**DW 25mL/kg**	23.367± 1.640

Groups	Body weight(g)	Groups	Body weight(g)
Post BCG day 14		**Post BCG day 21**	
ZZM 200mg/kg+BCG	23.775± 3.421	**ZZM 200mg/kg+BCG**	23.775±2.189
ZZM 100mg/kg+BCG	23.575±2.645	**ZZM 100mg/kg+BCG**	24.575±1.744
ZZM 50mg/kg+BCG	25.920±2.609	**ZZM 50mg/kg+BCG**	26.500±1.208
ZZM 25mg/kg+BCG	24.767±2.337	**ZZM 25mg/kg+BCG**	25.917±2.062
Imip 20mg/kg+BCG	24.633±1.052	**Imip 20mg/kg+BCG**	25.283±1.053
DW 10mL/kg+BCG	18.900±0.522	**DW 10mL/kg+BCG**	19.167±0.516
DW 25mL/kg	24.417±1.357	**DW 25mL/kg**	25.667± 0.804

There was a statistically significant simple effect of treatment between the groups $F(6,30) = 2.43, p<0.049$.However,there was no statistically significance simple effect between the groups $F(6,30)= 1.57, p<0.190$ at Pre BCG day 2.Similarly, there was no statistically significance simple effect of time(Pre BCG day 3,4) between the groups $F(6,30)=0.88, p<0.522$ and $F(6,30)=1.80, p<0.133$ respectively.

In addition, there was statistically significant simple effect of time (Pre BCG day5, Innoculation day 0, Post BCG day 1,Post BCG day2, Post BCG day3, Post BCG day4, Post BCG day5, post BCG day7, Post BCG day14, Post BCG day21.) between the groups $F(6,30)=2.65, p<0.035$,$F(6,30)=4.70, P<0.002$,$F(6,30)4.12, P<0.004$,$F(6,30)=2.80, P<0.0028$,$F(6,30)=3.30, P<0.013$,$F(6,30)=3.61, P<0.008$,$F(6,30)=3.33, p<0.0012$,$F(6,30)=4.62, p<0.002$ respectively.

Groups	Body weight(g)
ZZM 200mg/kg+BCG	22.519±1.157
ZZM 100mg/kg+BCG	22.723±1.083
ZZM 50mg/kg+BCG	21.264±0.968
ZZM 25mg/kg+BCG	22.819±0.885
Imip 20mg/kg+BCG	22.170±0.885
DW 10mL/kg+BCG	20.581±0.885

DW 25mL/kg	21.123±0.883

There was no statistically significant simple effect of time within group1,F(13,390)=1.54,p<0.100, moreover, there were statistically significant simple effect of time within the following groups(2,3,4,5,0and7),[F(13,390)=2.76,p<0.001,F(13,390)=10.26,p<0.000,F(13,390)=10.06,p<0.000,F(13,390)9.55,p<0.000,F(13,390)=4.36,p<0.000,F(13,390)=13.38,P<0.000 respectively.

Table4 Effect of methanol leaf extract of *Ziziphus Mauritiana*on body temperature (febrile response).

Time	**Temperature**
Pre BCG I hour	36.693±0.118*
Pre BCG 2 hours	36.897±0.104*
Pre BCG 3 hours	37.259±0.132*
Post BCG 4hours	37.713±0.104
Post BCG 5hours	37.439±0.116
Post BCG 1 day	37.225±0.139*
Post BCG 2days	37.196±0.094*
Post BCG 3 days	37.013±0.112*
Post BCG 4 days	37.114±0.109*

* That means the mean difference is statistically significant when compared with the innoculation time (4hour),p<0.001.

Simple effect of treatment within the level of time on body temperature due to BCG induction.

There was no statistically significant simple effect of time (pre BCG1hour) between the groups. F(6,34)=1.32,p<0.25.However, there was statistically significant simple effect of time at (pre

BCG2 hours) F(6,34)=3.72 p<0.006,pre BCG3 hour F(6,34)=3.34 p<0.01, inoculation time (post BCG 4 hours),F(6,34)=9.49 p<0.000,post BCG 5 hours, F(6,34)=12.47,p<0.000,post BCG day 1,F(6,34)=11.05,p<0.000,post BCG day2 (6,34)=18.52,p<0.000,post BCG day3,F(6,34)=11.55,P<0.000,Post BCG day4,F(6,34)=13.14,p<0.000.

There was statistically significant simple effect of time, this implies time has an effect on body temperature irrespective of the treatment group [F (5.889, 200.243)=12.311p<0.000.

Main effect of treatment

There was statistically significant main effect of treatment group on body temperature F(6,34)=13.402,p<0.000 and this reveal that there was significance difference between the groups

Groups	Body temperature(0c)
ZZM 200mg/kg+BCG	37.502±0.235
ZZM 100mg/kg+BCG	37.331±0.215*
ZZM 50mg/kg+BCG	37.248±0.215*
ZZM 25mg/kg+BCG	36.783±0.215*
Imip 20mg/kg+BCG	37.020±0.215*
DW 10mL/kg+BCG	38.467±0.215
DW 25mL/kg	35.852±0.215*

Table 5 Effect of methanol leaf extract of *Ziziphus mauritiana* onSucrose preference test

Time	Mean ±
Day 1	54.011±1.045
Day7	56.643±1.624
Day 21	56.586±1.101

Treatment group

Groups	Mean ±
ZZM 200mg/kg+BCG	58.417±1.859*
ZZM 100mg/kg+BCG	51.267±1.859
ZZM 50mg/kg+BCG	54.342±1.859*
ZZM 25mg/kg+BCG	64.400±1.859*
Imip 20mg/kg+BCG	60.942±1.859*
DW 10mL/kg+BCG	45.144±1.859
DW 25mL/kg	55.725±1.859*

*There was statistically significant difference between the DW+BCG group with all the group except 100mg/kg +BCG.

*Therefore the mean difference was statistically significant when compared with the DW+BCG $p<0.000$, except at 200mg/kg+ BCG which was not significance $p<0.099$.

*Group x time (interaction)

There was a statistically significance difference of time within the treatment group $F(8,272)=7.30, p<0.000$, group(2)$F(8,272)=2.88, P<0.004$, group(3)$F(8,272)=4.86, p<0.000$, group4, $F(8,272)=5.08, p<0.000$, group5,$F(8,272)=3.21, p<0.000$, group6$(8,272)=11.88, p<0.000$.

However, there was no statistically significance difference of time within the group7,$F(8,272)=1.88, p<0.064$.