INVESTIGATIONS OF ACTIVITY OF PHENOBARBITONE IN MICE MODELS OF DEPRESSION

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A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES, AHMADU BELLO UNIVERSITY, ZARIA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE DEGREE IN HUMAN PHYSIOLOGY

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MAY, 2017

DECLARATION

I hereby declare that the work in this dissertation entitled "Investigations of Activity of Phenobarbitone in Mice Models of Depression" was performed by me in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, under the supervision of Drs. R.A Magaji and M.G. Magaji.

The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this thesis was previously presented for another degree or diploma at any university.

Faruk, FARUK	_____	_____
Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "Investigations of Activity of Phenobarbitone in Mice Models of Depression" by Faruk FARUK meet the regulations governing the award of the degree of Masters of Science (M.Sc.) degree in the Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, under the supervision of Drs. R.A Magaji and M.G Magaji. It is therefore approved for its contribution to knowledge and literary presentation.

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DEDICATION

All Praises and glory belongs to Allah (S.W.T) the Lord, Cherisher and Sustainer of all the worlds, May the Peace and Blessings of ALLAH be upon the last and Noble Messenger, Muhammad (S.A.W) and His entire household.

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Abstract

Depression is a severe neurological condition that interferes with our brain neurochemistry and synaptic processes, ultimately leading to intense expression of our emotions. Major fallouts observed from the use of antidepressant including high rates of remission, impotency and suicidal tendencies led to researches to screen other compounds with antidepressant potentials. Various studies have highlighted the antidepressant potential of GABA mimetics such as Barbiturates and Benzodiazepines. This study investigated the antidepressant potential of Phenobarbitone in mice models of depression. A total of 108 Adult male Swiss Albino mice were used throughout the study. The study was conducted in two phases: an acute and a chronic phase respectively. Each mouse received a single intraperitoneal injection of Phenobarbitone 0.5 mg/kg, 2.5 mg/kg, 5 mg/kg and 10 mg/kg respectively. Imipramine 20mg/kg served as the standard drug while distilled water (10 ml/kg) was used as the vehicle. The mice were subjected to a series of stressors using the Forced swimming test (FST), Tail suspension test (TST) and Chronic mild stress (CMS) models of depression, and the duration of immobility in the TST and FST were taken as a sign of behavioral despair, while decrease in sucrose preference was taken as sign of anhedonia, which are core symptoms of depression. The acute phase of the study lasted for just a day while the chronic phase lasted for 30 days. A statistical significant decrease in mean immobility time was observed at the highest dose tested i.e. 10 mg/kg treatment group ($p<0.05$) when compared to control in both phases of the study i.e. Acute phase: TST126.1 ± 11.34, FST54.5 ± 7.34*, Chronic Phase: FST54.5 ± 7.34* and SPT 24.5 ± 1.06*. The mice were further subjected to the open field (OFT) which showed no significant increase in locomotory activity (line crossings) in all the treatment groups when compared to control, this indicates that the results obtained from both phases of the study was due to antidepressant potential of Phenobarbitone and not due to increase in locomotory or due to the stimulant effect of the Phenobarbitone. This study shows that phenobarbitone possess significant antidepressant potential at 10 mg/kg group ($p<0.05$) when compared to control in both phases of the study.*

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LIST OF ABBREVIATIONS

ANOVA	Analysis of Variance
5HT	5-hydroxytryptamine (Serotonin)
ACTH	Adrenocorticotrophic hormone
AKT	Protein Kinase B
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic receptor
Arc	Activity regulated cytoskeletal-associated protein
ATP	Adenosine triphosphate
BDNF	Brain Derived Neurotrophic Factor
Ca++	Calcium ion
CNS	Central Nervous System
CREB	Camp response element binding protein
dlPFC	Dorsolateral prefrontal cortex
DNA	Deoxyribonucleic acid
DSM-V	Diagnostic and Statistical Manual of Mental Health Criteria Five
Eef2k	Erythrocyte elongation factor 2k
fMRI	Functional Magnetic Resonance Imaging
GABA	Gamma Amino Butyric Acid
$GABA_A$	Gamma Amino Butyric Acid A Receptors
$GABA_B$	Gamma Amino Butyric Acid B Receptors
$GABA_C$	Gamma Amino Butyric Acid C Receptors
GIRK	G-protein-regulated inward-rectifying K^+ -channels
Glu-R	Glutamate receptor

CHAPTER ONE

1.0 Introduction

Depression is a severe neurological condition that interferes with the brain neurochemistry and synaptic processes, ultimately leading to intense expression of our emotions. This is evident from studies on neural tissues and grey matter of most patients suffering from depression and other forms of neurological anomalies (Xiao-Li *et al*., 2015). Severe forms of depression also lead to the atrophy of vital brain regions that are involved in the regulation of mood and behaviour such as the limbic system, prefrontal cortices and the hippocampus (Li *et al*., 2015).

Depression is characterized by a myriad of clinical symptoms including generalized decrease in mood, behavioural despair, melancholia, loss of interest in previously enjoyed activities (anhedonia) and a decrease in cognitive and motor skills which becomes persistent for at least two or more weeks. Severe depression leads to constant suicidal ideation or possible suicide, according to the diagnostic and statistical manual of mental health DSM-V criteria as edited by the American Psychiatric Association (APA) (Mayes and Horwitz, 2005).

Depressive episodes have been broadly classified into unipolar and bipolar depression. There is a very little difference between the two types of depression and often times they occur together (Daniel and Nick, 2011). Unipolar depression is mostly seen in normal physiological without an underlying pathological condition or mania, such as during pregnancies, in adolescents, ultradian rhythms or due to chronic stress and other metabolic diseases such as diabetes and hypertension (Jonathan and Mark 2012).

Bipolar depression on the other hand presents clinical symptoms of depression with mania and psychoses. Studies have shown that it is mostly comorbid with most psychiatric illnesses and is often accompanied by manic episodes, distorted thoughts and hallucinations (Driessen, *et al*., 2010).

In 2011, the World Health Organization (WHO) ranked depression as the fourth leading cause of morbidity and by the year 2020, it is projected to be the main cause of disease burden worldwide (Jean and Mike, 2011). Depression leads to a significant decrease in the quality of life of most patients and hinders interpersonal relationships, and work output per person, thus leading to significant impairment in day to day activities (Anita *et al*., 2012).

Globally, over 120 Million people are affected with depression and studies have shown that the lifetime prevalence of depression is within range of 10% to 15% per individual (W.H.O, 2015). Depression can occur at any age and studies have shown that females are more prone to depression than men. Even though the reason for the high incidence of depression in females is not yet fully understood, but hormonal variations are thought to play a major role in these findings (Alize *et al*., 2010).

Studies also showed that people from the Northern part of Africa have the highest rate of depression in the continent, and this has been attributed to political instability, conflict, poverty, and low income amongst most skilled and unskilled workers in those regions (Alize *et al*., 2010).

In Nigeria, a study carried out by Yusuf and Adeoye (2011) on the prevalence of depression among civil servants in Osun state revealed that poor remunerations, increasing job demands and unsuitable working conditions are the chief causes of depression among civil servants in the state. In a similar research by Lasebikan *et al.,* (2012), it was found out that over 50% of patients attending primary health care services in Lagos Island have depressive symptoms.

Most of the orthodox antidepressants have proven effective in the management of depression, though they are associated with some adverse effects. These include the monoamine oxidase inhibitors (MAOIs), tricyclic antidepressants (TCAs), specific serotonin receptor inhibitors (SSRIs) and more recently the specific Norepinephrine receptor Inhibitors (SNRIs) (Osby *et al*., 2001).

The wide range of side effects observed from the use of most orthodox antidepressants led scientists to venture into research on some compounds with promising antidepressant potentials and relatively fewer side effects (Lorain, 2003). Some of these observed side effects observed include sexual dysfunction, sedation, remission and suicidal tendencies after prolonged usage (Ferguson, 2001).

Studies on drugs that act via the GABA-Benzodiazepine receptor complex have been shown to possess some antidepressant potentials (Chadi *et al*., 2015). Furthermore, compounds that act via the NMDA glutamate receptors such as ketamine, lithium and memantine have shown to some antidepressant potentials by enhancing synaptic processes, neuroplasticity and increasing the brain levels of neurotrophic proteins such as the brain derived neurotrophic factor (BDNF) and nerve growth factors (NGF) (Anita and Lisa, 2012)

1.2 Statement of Research Problems

Depression is a debilitating medical condition with severe neurological consequences. It contributes significantly to the global burden of disease and affects the quality of life of most depressed patients their work output and interpersonal relationships. It contributes significantly to the disability adjusted life years per person, accounting for the highest rate of suicidal cases worldwide (WHO, 2015).

The impact of depression is felt amongst a wide range of population, and results in negative socio-economic effects. Depression can occur at any age and is mostly comorbid with most neuropsychiatric illnesses (Melartin *et al*., 2002); and oftentimes most patients suffering from depression are not aware of their condition (Mijung and Jürgen, 2011).

Moreover, most of the drugs used in the management of depression are associated with a host of side effects and often times patients find it hard to conform to regular drug regimen. A study by Janos (2007) showed that the success rate for most of the known antidepressant is less than 60% and more than 45% of patients taking antidepressants do not show improved therapy even after advanced treatments (Janos, 2007).

In a similar study by Khalid, (2012) showed that about 15% of all patients taking antidepressants do not respond to treatment at all and about 30% show high relapse rate (Khalid, 2012). Hence intensive researches into newer compounds with antidepressant potentials are needed in the search for a more efficacious antidepressant with relatively fewer adverse effects.

1.3 Justification of the Study

Most of the data emanating from studies on CNS depression are quite alarming and some of the current drugs used in the management of depression are plagued with a large side effects profile. Studies have shown that severe depression leads to a decrease in cortical cell volumes and subsequent atrophy of vital brain regions involved in the regulation of mood and emotions such as the limbic system, hippocampus and the prefrontal cortices.

Phenobarbitone is a long acting barbiturate used in the management of epileptic seizures (Martin, 2012) and as an adjuvant in anaesthesia (Markus, 2011). Phenobarbitone is a positive allosteric modulator of the $GABA_A$ receptor subtype which binds at the beta subunit (a distinct

binding site from other allosteric modulators of GABA such as benzodiazepines) to elicit its potent CNS depressant effect (Czapiński *et al*., 2005).

Studies have shown that drugs that act via the Benzodiazepine-GABA receptor complex such as barbiturates and benzodiazepines posses some antidepressant properties (Chadi *et al*., 2015; Abdallah *et al*., 2014). Thus this work aims at investigating the antidepressant potentials of phenobarbitone at varying doses.

1.4 Aim and Objectives of the Study

1.4.1 Aim

The aim of this study is to investigate the antidepressant potential of phenobarbitone in Murine models of depression.

1.4.2 Objectives of the study

i. To establish the antidepressant effect of Phenobarbitone in the Forced swimming test model of depression

ii. To establish the antidepressant effect of Phenobarbitone in the Tail suspension test model of depression

iii. To establish the antidepressant effect of Phenobarbitone in the Sucrose preference test model of depression

iv. To establish the antidepressant effect of Phenobarbitone in chronic mild stress model of depression

1.5 Research Hypothesis

Phenobarbitone does not possess significant antidepressant effect in acute and chronic stress models of depression in mice.

CHAPTER TWO

2.0 Literature Review

Depression is a severe neurological condition in which some of our emotions are experienced very intensely. Although comorbid with most psychiatric illnesses, depression is seen in some physiological conditions such as in post-menopausal women, severe pains, adolescents and in some disease conditions such as diabetes and organ failure or other terminal illnesses (François *et al.*, 2010).

Antidepressants are drugs used in the management of depression and other related disorders such as generalized anxiety disorders and panic attacks (Chaitra *et al*., 2012). These drugs range from the first generation antidepressants such as the monoamine oxidase inhibitors (MAOI) to the more recent antidepressants such as the specific serotonin receptor inhibitors (SSRI) and serotonin-norepinephrine reuptake Inhibitors (SNRIS) (Chaitra *et al*., 2012).

Recent studies have shown that some drugs that act via the glutamatergic and gabergic systems possess some antidepressant properties. Furthermore, studies have shown that both agonists and allosteric modulators of the Benzodiazepine-GABA receptor complex such as ketamine, lithium, and memantine possess remarkable antidepressant properties (Chadi, 2015).

Disruption in the body internal clock (biological rhythm) has also been linked to major depressive disorders and early preclinical findings have shown the rapid antidepressant potential of the melatonin receptor agonist such as Agomelatin (Hickie and Rogers, 2011). This drug acts by binding to M1and M2 receptors of melatonin and selectively antagonising 5-HT_2C receptors, thereby up regulating the cathecolamines at the synapses (Blanca, 2014). Thus melatonin agonists such Agomelatine resynchronise the body circadian rhythm and alleviates depressive symptoms (David *et al*., 2014).

Generally, antidepressants functions to alleviate depressive symptomology by increasing the availability of endogenous biogenic enzymes such as the monoamines: serotonin, norepinephrine and dopamine across the synapses in the brain. Hence, a decrease in the amount of neurotransmitters at the synapse has been linked to depression (Laura, 2011).

The older generation antidepressants comprised of the monoamine oxidase inhibitors and included drugs such as isoniazid, iproniazid, phenelzine and selegeline. This group of drugs catalyzes the deamination of intracellular monoamines and elevates the level of serotonin and dopamine in the body by inhibiting the enzyme monoamine oxidase. But they are associated with some adverse effects including sleep disturbances, muscle wasting and tyramine crisis or hypertensive crisis (Jess and Karen, 2000).

Major fallouts observed from the use of this antidepressant led to the discovery of the second generation antidepressants in the late 1950's. These drugs had more efficacies and less side effect profile, thus the tricyclic antidepressants (TCAs) became the candidate of choice. Drugs in this category include Imipramine, amitriptyline, doxepine etc (Chaitra *et al*., 2012).

Tricyclic antidepressant (TCAs) increases the level of the catecholamine at the synapses, especially that of Norepinephrine (NE) and to a lesser extent serotonin (5HT) across most of the synapses in the brain. The tricyclic antidepressants also allow for down regulation of the post synaptic receptors of NE and 5HT, so that these neurotransmitters will be available at the synapses for a longer period of time (Gillman, 2007).

The tricyclic antidepressants have a large number of receptors in the body, therefore are associated with a wide range of undesirable effects. Tricyclic antidepressants act on M1 muscarinic receptors to cause its anticholinergic effects, such as dry mouth, impotence and impaired vision (Agnes, 2010). It also acts on histamine H1 receptors to cause weight gain and sedation. On adrenergic receptors, tricyclic antidepressants have been shown to cause postural hypotension (orthostatic hypotension) (David *et al*., 2012).

Overtime, most of the old generation antidepressants lost patronage due the number of undesirable effects associated with them. Thus, researchers focused on compounds with promising antidepressant activities and relatively fewer adverse effects. Therefore researches led to the formulation and synthesis of the specific serotonin reuptake inhibitors (SSRIs). This group of drugs have better therapeutic indices with relatively fewer side effects profiles compared to the TCAs. Furthermore, most of the side effects of associated with the SSRIs were found to be dose-dependent and can be attributed to serotonergic effects (David *et al*., 2012).

The specific serotonin receptor inhibitors (SSRIs) works primarily by increasing the level of serotonin in the brain, and unlike the tricyclic antidepressants do not affect the level of norepinephrine in the brain. Though the exact mechanism of action of the SSRIs remains uncertain, but Ser-438 residue in the human serotonin transporter (hSERT) appears to be a

determining factor in SSRI potency (James and Ferguson, 2001). Examples of drugs under this category include Paroxetine, Citalopram, Flouxetine and Sertraline (James and Ferguson, 2011).

Later on the serotonin-norepinephrine reuptake inhibitors SNRIs were synthesised and they showed better therapeutic potentials in the management of depression than the preceding medications such as the TCAs. The SNRIs had similar mechanism of action to the SSRIs, they work by increasing the levels of serotonin and epinephrine in the synapses with sparing selectivity (James and Ferguson, 2001).

Both serotonin and norepinephrine are produced at the synapses and are usually reabsorbed by the nerve terminals that produced them, but SNRIs prevent the active reuptake of these neurotransmitters, thereby maintaining their concentration at the synapse for a longer period of time. Drugs under this category include venlafaxine and duloxetine (Gregory and Irwin, 2012).

2.1 Areas of the Brain Implicated in the Pathophysiology of Depression

Studies using functional magnetic resonance imaging (fMRI) and histology studies of post mortem brain tissue have highlighted some of the areas in the brain that are involved in the pathogenesis of depressive disorders. Most of these brain regions are located in the frontal lobe and they include: the prefrontal cortex, anterior cingulate cortex, ventromedial cortex, orbitofrontal cortex, limbic system and the hippocampus. Depression leads to structural and functional changes in the brain cytoarchitecture, altering neurogenesis and protein translation (Michael and Diego, 2014).

Depression affects most of the vital centres in the brain that are involved in the regulation of mood and behaviour, thence giving rise to stereotypic phenotypes seen in depressed patients such as anhedonia and emotional despair (Drevets, 2007). Depression affects the nerves and glia cells in brain, decreasing their integrity, synaptic capabilities and morphology, therefore limiting their functions (Shelime, 2011). It also affects transmission of impulses and functions of neuronal populations, and reduces the number of neuronal circuitries. Depression also leads to a decrease in cortical cell volume especially in the limbic system and the hippocampus.

This leads to further alteration in the neuronal circuitries, affecting synaptic plasticity, neurogenesis and protein translation (Duman and Aghajanian, 2012). It also limits complex cognitive behaviours which eventually modify an individual's character by displaying array of

symptoms including negative emotional responses, poorly regulated anger, frustrations and intolerance (Drevets, 2007).

Studies have shown that depression affects the prefrontal cortex and its dense connections with other areas of the brain that are associated mainly with the regulation of mood and behaviours such as the hypothalamus and limbic system. Both the dorsolateral and ventromedial regions of the prefrontal cortices are affected with depression and their volumes have been shown to decrease in size overtime due to this condition (Xiao-Li *et al*., 2015).

Different researches have shown that the ventromedial prefrontal (VmPC) cortex is concerned with emotional and affective behaviours and dysfunction of this brain region with its complex connections with the hypothalamus and the periaqueductal grey matter is implicated in major depression (Michael and Jordan, 2009).

The dorsolateral prefrontal cortex (dlPFC) has also been implicated in the pathogenesis of depressive disorders. This brain region has dense connections with the premotor area and other specific sensory cortices. Imaging studies have shown a wide range of decreased activity in depressed patients at rest with a decreased blood flow and glucose metabolism. Administration of antidepressants have been shown to cause an increase in the activity of the brain Xiao-Li *et al*., (2015) and reverse the decrease in the size and volumes of most areas of the brain affected during depression (Amy *et al*., 2016). The figure in the next page gives a general outline of the areas of the brain involved in the pathogenesis of depression.

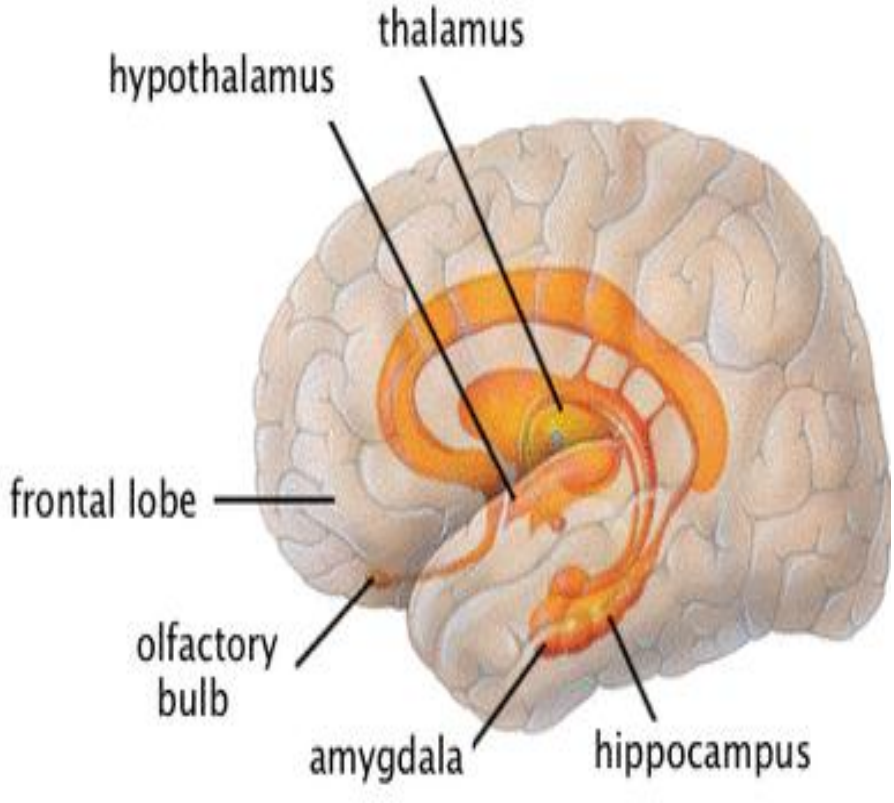

Figure 1: Areas of the brain involved in the pathogenesis of depression (Amy *et al*., 2016).

2.2 Major Hypothesis of Depression

2.2.1 The monoamine hypothesis of depression

The monoaminergic theory of depression postulates that a decrease or alterations in monoaminergic transmission especially of serotonin and norepinephrine plays a great role in the aetiology of most depressive disorders (Michael and Charles, 2004). Monoaminergic pathways

are highly responsive to aversive stimuli and precipitate depressive symptoms in response to noxious stimuli such as stress (Krishnan and Nestler, 2008).

Alterations in the monoaminergic pathways by noxious stimuli such as stress cause the brainstem to release norepinephrine to the limbic system via the action of the locus coerulus. The limbic system in turn causes the amygdala and paraventricular nucleus of the hypothalamus to release corticotrophin releasing factor (CRF), which in turn stimulates anterior pituitary gland to release (ACTH) (Femina *et al*., 2001).

Adrenocorticotrphic hormone (ACTH) stimulates Zona fasciculata of the adrenal gland to secrete cortisol which is needed by the body for metabolic activities and responses to stress. A constant negative feedback mechanism is therefore needed to inhibit the hypothalamus from secreting excess cortisol, but this negative feedback mechanism is absent in depressed patients and accounts for the high level of cortisol seen in most depressed patients (Femina *et al*., 2001).

Dysregulation in the monoaminergic pathways and up regulation in the plasma level of cortisol serves as a major biomarker for the treatment of depression. Elevated plasma levels of cortisol lead to a significant decrease in the blood levels of monoamines such as serotonin and epinephrine as seen in most patients with major depressive disorders. And this has been attributed to the over stimulation of the hypothalamo-pituitary adrenal axis (HPA) and consequent decrease in the production of the monoamines (Femina *et al*., 2001).

The raphe nuclei in the brainstem secrete serotonin which decreases the amygdale-stress response from the nucleus coeruleus and other brain regions such as the hippocampus and prefrontal cortices. Hitherto stored memories of the aversive stimuli in the hippocampus are also decreased, thus further limiting the excitation of this pathway, dysregulation in the excitatory/inhibitory inputs to this pathway aggravate depression (Kerry and Charles, 2000).

Understanding the monoamine hypothesis of depression has paved way for the development of different classes of drugs with antidepressant potentials (Margaret *et al*., 2012). Different classes of drugs that either delay the active reuptake of monoamines from the synapse or further delay the dissociation of the neurotransmitters in the synapse by blocking their active reuptake have been used as antidepressants to management of depressive episodes (Margaret *et al*., 2012).

Drugs such as the Monoamine oxidase inhibitors, tricyclic antidepressants (TCAs) and tetracylic antidepressants have been used in the management of depressive disorders. These drugs decrease the active reuptake of serotonin and adrenaline from the synapse, making them available for a longer period of time. Newer antidepressants such as the specific serotonin receptor inhibitors (SSRI) decrease the action of serotonin transporters (SERT) in the synapse (Margaret *et al.,* 2012).

2.2.2 The cytokine hypothesis of depression

The cytokine hypothesis of depression implicates the pro-inflammatory cytokines in the pathophysiology of depression. Stress has been shown to prepare the body against potentially dangerous situations, but this leads to the over stimulation of the hypothalamo-pituitary adrenal axis and release of inflammatory biomarkers (Allen and Annells, 2010).

Thus stress changes the body's response to peripheral inflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor alpha (TNF-α). Inflammatory cytokines promote the differentiation of lymphocyte, enhances the phagocytic ability of the lymphocytes and increases vascular permeability (Müller *et al*., 2011) thereby enhancing immunity.

Studies have also shown that most neurological illnesses are accompanied by high level of serum inflammatory biomarkers including interleukin-1 (IL-1), interleukin-2 (IL-2), interleukin-6 (IL-6), gamma interferon (IFN-λ) and Tumour necrosis factor alpha (TNF-α) (Jennifer *et al*., 2010).

Cytokines are important immunomodulators and have been implicated in some physiological mechanisms within the body such as neurogenesis and synaptic plasticity, hitherto over expression of these inflammatory cytokines interferes with some physiological mechanisms in the body, eventually leading to neurological conditions such as depression and anxiety (Jennifer *et al*., 2010).

Cytokines plays a vital role in the pathogenesis of depression and other depressive disorders by altering the expression of neurotransmitters, synaptic processes and neuroendocrine responses (Amisha, 2013). Inflammatory cytokines also provokes the kynurenine pathways which lead to shunting of the production of serotonin, thus further implicating the cytokines in the aetiology and pathogenesis of depression. The level of most inflammatory biomarkers is seen to rise in most depressed patients and other neural conditions, thus giving an insight into link between the levels of inflammatory cytokines to most neural disorders (Müller *et al*., 2011).

2.2.3 The circadian rhythm hypothesis of depression

Circadian rhythm is the body internal clock that regulates and entrains our physiological rhythms to a 24hr cycle. This includes the sleep/wake cycle, core body temperature, emotions, hormonal secretions, cognitive functions and other physiological parameters. Most depressive illnesses are linked to a dysregulation in this internal biological clock which regulates our daily activities (Philippe and Emilie, 2012).

The light that enters our eyes permits the sensory capacity of vision by stimulating photoreceptive pigments in the retina such as melanopsin (Kennaway, 2010). Visual signals are sent to the posterior lobe (visual center) for interpretation. But along the visual tract a tract goes to the suprachiasmatic nuclei (SCN) were it analyzes the light signal and interpret the length of the day and night in order to entrain and synchronize the body to a twenty four hour cycle (Kennaway, 2010).

Dysregulation in the body's internal clock has been implicated in the aetiology of most depressive disorders because it produces symptoms similar to what is seen in most depressive disorders, including disruption in the sleep wake cycle, changes in hormonal levels, (especially levels of cortisol) and altered regulation of mood and behaviour (Blanca, 2014).

Melatonin agonist Agomelatine has shown remarkable antidepressant potentials (Millan *et al*., 2003). This drug attempts to resynchronise the body's biological rhythm by binding to the MI and M2 receptors of melatonin in the brain, as well as serving as partial antagonist for the 5HT2c receptors. This action temporarily inhibits serotonin and increases the biogenic enzymes at the synapses (dis-inhibition), thus alleviating depressive symptoms at aligning the body's internal rhythm of the 24hr cycle (Blanca, 2003).

Phototherapy has been used in management of circadian rhythm anomalies and has proven effective in the management of depression and other neuropsychiatric illnesses, since it attempts to realign the body endogenous circadian clock with the 24 hour duration of our sleep wake circle (Golder and Macy, 2011).

2.2.4 Role of the kynurenine pathways in the pathophysiology of depression

The kynurenine pathway is the major pathway for tryptophan catabolism, but tryptophan serves as the major precursor in the synthesis of serotonin and melatonin, and deficiency of serotonin is implicated in aetiology of many neurological illnesses such as depression and anxiety (Ian and Aimin, 2015). Activation of the kynurenine pathway diverts available tryptophan needed for the synthesis of serotonin, this leads to a reduced level of serotonin in the brain synapses and up regulation of tryptophan metabolites and other neurotoxins, thus exacerbating neurological illness (Oxenkrug, 2010).

Activation of the kynurenine pathway shunts the production of serotonin and hence increases tryptophan catabolism. This in turn leads to the accumulation tryptophan other metabolites in the liver such as tryptophan 2, 3-dioxygenase (TDO), Kynurenine-3-monooxygenase (KMO), 3-hydroxykynurenine (OHK) and 3-hydroxy anthranilic acid 3-HAA. The kynurenine pathway also leads to the production adenosine triphosphate (ATP), picolinic acid (PLC) and nicotinamide dinuclotide phosphate (NAD) (Gislaine, 2015; Maria *et al*., 2016). Studies have shown that TDO worsens depressive states and increases the number of pro inflammatory biomarkers and down regulates NMDA glutamate receptors (Heng *et al*., 2016).

Furthermore, studies have shown that many antidepressants act on the kynurenine pathways to elicit their antidepressant actions. Thus the antidepressants properties of sertraline (an SSRI) have been shown to reduce the shunt on the kynurenine pathway and increase the cellular levels of serotonin (Zhu *et al*., 2013). This plays a significant role in the management of depression and subsequent reduction of its deleterious properties (Heng *et al*.,

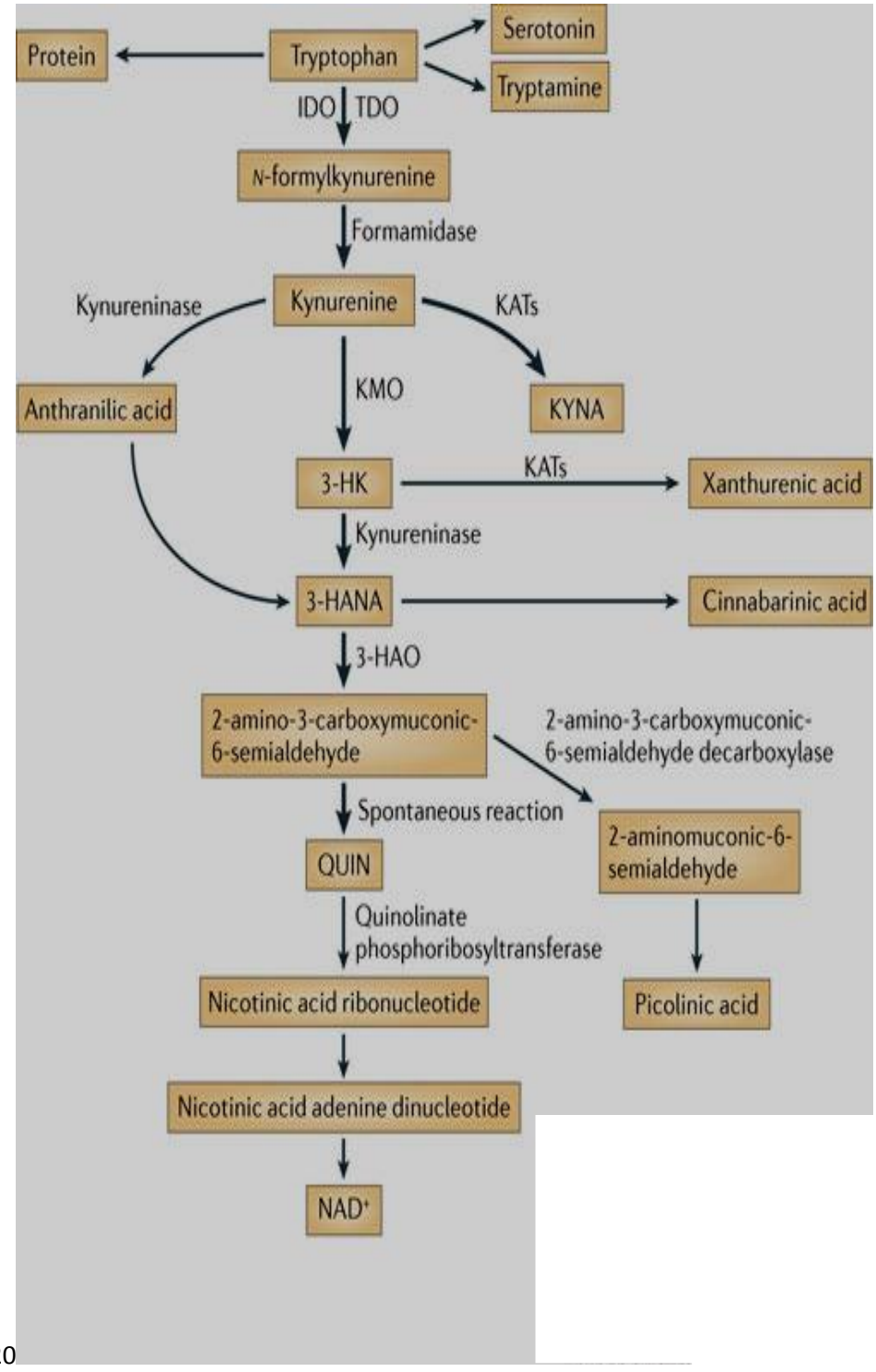

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Figure 2: Kynurenine signalling cascades (Gislaine *et al*., 2015).

2.2.5 Neurotrophines and their roles in the pathophysiology of depression

Neurotrophines are proteins that are synthesized in the brain and are associated with a host of activities including synaptogenesis and protein translation (Nagakawa *et al*., 2005). The neurotrophines are one of the most prevalent growth proteins and have been shown to promote growth and survival of most neurons (Nagakawa *et al*., 2002). A study carried out by Li *et al.* (2015) showed that neurotrophines possesses antidepressant properties and co-administration with other antidepressants such as the specific receptor inhibitors (SSRI) has shown a synergistic effect.

The brain derived neurotrophic factor acts on various signalling pathways which lead to axonal growth and survival. It binds to its high affinity receptor (TRKB receptors) and facilitates long term potentiation (LTP) via sustained TRKB mediated CREB activation, which binds to CREB sites in the BDNF promoter by converting long term potentiation to late long term potentiation (Tao *et al*., 1998).

Reduction in the brain levels of BDNF have been linked to most depressive disorders since it leads to reduced efficiencies in synaptic connections and consequently dendritic atrophy (Hui and Zhe-yu, 2015). Transgenic mice of the BDNF gene have also shown developmental anomalies and synaptic dysfunctions, and often time experimental mouse lacking the BDNF gene dies immediately after birth (Fani *et al*., 2011).

Advances in neuronal research on the role of brain derived neurotrophic factor (BDNF) as an antidepressant in the management of depression led scientist to venture and test other compounds and that have shown promising antidepressant properties. Studies on ketamine (a non-competitive antagonist of the post ganglionic NMDA receptors) have shown rapid and sustained antidepressant properties via increased neurogenesis and synaptic plasticity (Lisa and Carlos, 2015).

Clinical trials using a large double blind study has shown that intravenous administration of sub anaesthetic dose of ketamine (0.5 mg/kg) over a period of 40 minutes can alleviate depressive episodes even on treatment resistant patients (TRP) (Caroline *et al*., 2014). The antidepressant response that follows acute administration of ketamine is very rapid and lasts for about two to three weeks (Lisa *et al*., 2015).

The mechanism underlying the rapid antidepressant effects of ketamine revolves around its antagonism of the NMDA glutamate receptors, subsequent up regulation of glutamatergic transmission and the complex interaction with downstream neuronal pathways involving the TrKb receptors, mTOR Complex 1 (mTORC1), and inhibition of the Glycogen Synthase Kinase-3 (GSK-3) pathways, thus enhancing both protein translation and neuroplasticity (Li *et al*., 2010).

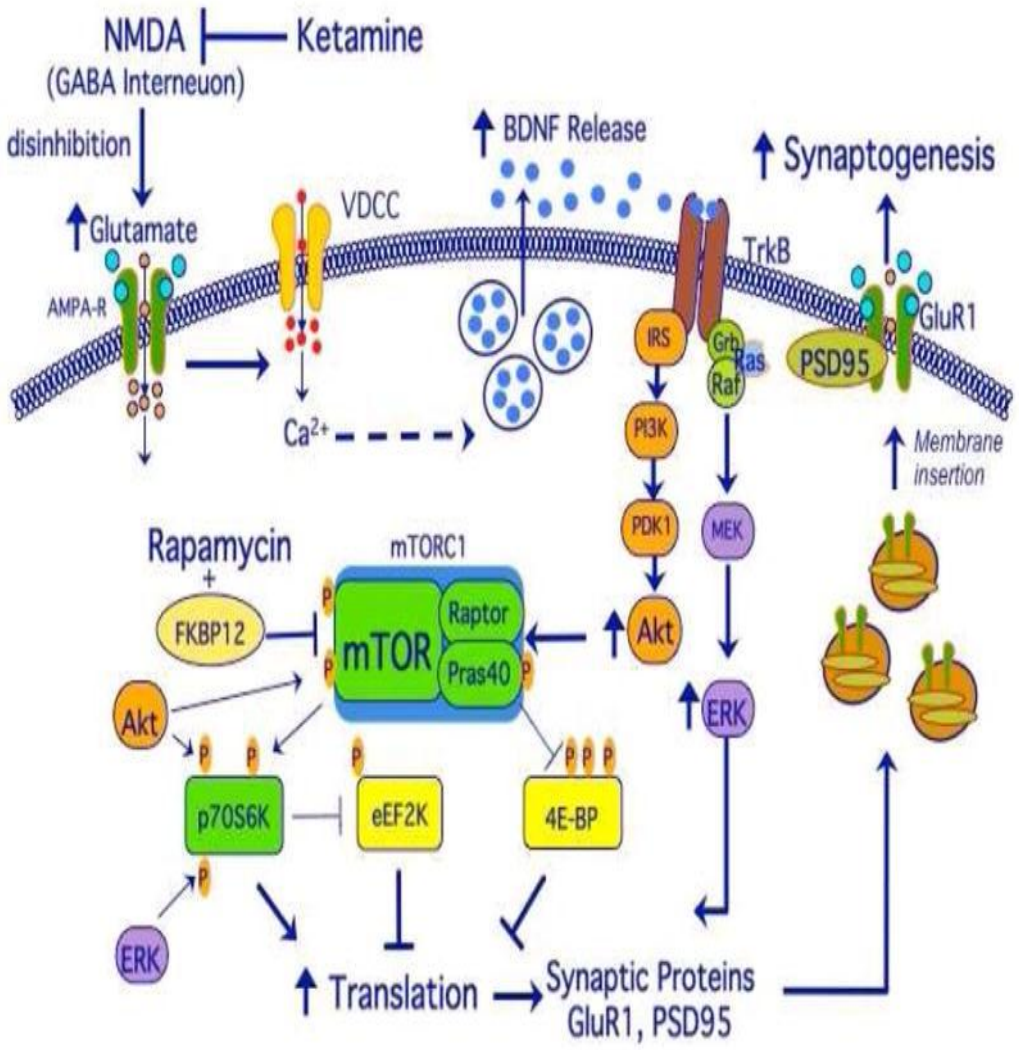

Figure 3: Signalling pathways involved in the fast antidepressant effects of ketamine and the potentiation by lithium, (Lisa *et al*., 2015).

2.2.6 The role of GABA in depression

Gamma amino butyric acid (GABA) is the chief inhibitory neurotransmitter in the mammalian central nervous system and constitutes about 40% of all synapses in the adult brain (Sudheer and Kuppast, 2012). Existing data from preclinical, clinical and post-mortem tissue studies have shown the link between GABA and depression. This is evident from studies on GABA interneurons and their role in mediating fast antidepressant properties of different allosteric modulators of the GABAergic system (Möhler, 2005; Cornelisse *et al*., 2007).

Gamma Amino Butyric Acid (GABA) is involved in some physiological processes in the body such as memory, cognition, vision and pain. Defect in GABAergic transmission is implicated in some neurological disorders such as depression, epilepsy and stroke (Pilc and Nowak, 2005). Most cell membranes in the CNS and other astrocytes expresses GABA receptors, and these GABA receptors decreases neuronal excitability via by increasing the chloride influx time and inducing inhibitory post synaptic potential (IPSP) (Pilc which Nowak, 2005) and hyperpolarises their cell membranes and reduce their excitability (Holly and Amy, 2004).

Summation of these induced IPSP's will lead to inhibition of conduction of action potential across the synapse via sustained hyper polarization of the post synaptic neurons, thence decreasing the tendency to generate action potentials by the post synaptic neurons, ultimately depressing the neurons (Holly and Amy, 2004). Positive allosteric modulators of $GABA_A$ receptors such as barbiturates and benzodiazepines act on this receptor, and elongate the duration of opening of the ion channel, thus enhancing the potency and efficacy of the receptors.

Studies carried out on the different GABA receptors showed some promising antidepressant potentials (Krystal *et al*., 2002) of the gabergic system. Antidepressants have been shown to increase the functions and activity of GABA receptors in laboratory animals, especially on $GABA_A$ and $GABA_B$ receptors in rodent's hippocampus and prefrontal cortices. A study by Slattery *et al.* (2005), showed an up regulation in $GABA_B$ receptor subtypes following acute treatment with antidepressants and their allosteric modulators.

Studies using different animal models of depression have also shown that GABA receptor subtypes are modified with chronic administration of antidepressants which have led researchers to postulate that depressive illnesses could be due to alterations in the synthesis of GABA in the brain (Anita *et al*., 2014). Furthermore, a study carried out by Li and co workers (Li *et al*., 2012) showed that allosteric modulators of GABA receptor such as benzodiazepines and barbiturates potentiate the antidepressant properties of the Gabergic system. This has been proposed to be via an increase in GABA mediated actions on its receptor subtypes and also by enhancing receptor responses to GABAergic system (Elena *et al*., 2015).

2.2.6.1 GABA receptors and their role in mediating fast antidepressant responses

The neurophysiologic role of GABA receptors is mediated via a complex interaction between its individual receptors and other neuromodulators (Martin, 2002). Hence, both the ionotropic and metabotropic GABA receptors have been implicated in the pathophysiology depression. The Gabergic system serves a major balance between neuronal inhibition and excitation and hence activation of the Gabergic system regulates different neurons and interneurons via its individual receptors i.e. $GABA_A$, $GABA_B$ and $GABA_C$ receptors respectively (Pantea *et al.,* 2014).

The ionotropic $GABA_A$ receptors include the $GABA_A$ and $GABA_C$ receptor subtypes are made of multiunit membrane proteins that bind to GABA and open intrinsic chloride ion channels (Marta and Francine, 2014). All members of this family consist of a pentameric structure surrounded by a central pore with two cysteine residues separated by two amino acid residues.

Sixteen different $GABA_A$ receptor subunits are currently known including: α1-6, β1-3, γ1-3, δ, ε, π and θ; and most of the $GABA_A$ receptors consists of two α, two β and two γ subunits. Extra synaptic GABA receptors have also been identified in some dendrites and cell bodies and have been shown to poses antidepressant potentials (Pantea *et al.,* 2014).

The $GABA_B$ metabotropic receptors on the other hand are heterodimetric G protein coupled receptors that elicits their effects via second messenger systems (Feyza and Cynthia, 2011). The $GABA_B$ receptors are indirectly coupled to K^+ channels, and when activated, enhances the G-protein-regulated inward-rectifying K^+-channels (GIRK) which cause a decrease in Ca^{++} conductance by increasing cAMP production via intracellular mechanisms that are mediated by G proteins, i.e. coupling of Gi and Go (Richard and Timothy, 2009).

Co-administration of GABA and other antidepressants such as the tricyclic antidepressants has shown remarkable potentiation of antidepressant property of the TCAs (Subroto *et al*., 2011). Both clinical and preclinical studies have also shown improved antidepressant potentials of GABA receptors following antidepressant treatment. Other studies showed that GABA receptors are modified with chronic antidepressant medications and their number is decreased significantly in depressed mouse models of depression (Freiling and Bleich, 2006).

Electrophysiological studies have also given a better insight on the antidepressant potentials of GABA and it receptors. Studies have shown that GABA increases the neuromodulation of its individual receptor subunits at the $GABA_A$ receptor subunits. Thus the $GABA_A$ receptor subunits α2 and α3 have been proposed to be novel target for antidepressant responses (Elif *et al*., 2013). Also, both preclinical and clinical and data suggested that modification of the $GABA_B$ receptor subunits may contribute to symptoms of major depressive disorders and could be possible targets for antidepressant responses (Subroto *et al*., 2011). Furthermore, post-mortem examination of the brain of suicide victims that took antidepressants revealed a change in gabergic neurotransmission with related changes in $GABA_A$ and $GABA_B$ subunit expression (Subroto *et al*., 2011). Thus this shows a strong relationship between the GABA receptors and depression.

2.2.6.2 *Role of barbiturates and other allosteric modulators of GABA receptor sub-type in mediating rapid antidepressant responses*

Barbiturates such as phenobarbitone and thiopentane are GABA analogues that are used in the management of epileptic seizures and as adjutants during anaesthesia in surgical procedures (Jean-Philippe, 2015). The receptors of barbiturates are widely distributed throughout the CNS and they exert their effects via its individual receptors (Jean-Philippe, 2015).

A study carried out by Imran *et al*. (2016) showed that agents that act via $GABA_A$ receptor subtypes such as the barbiturates may possess antidepressant potentials. This is evidenced by an increase in the mobility time in the FST and TST in mice models of depression. Furthermore, barbiturates potentiate both the pre and postsynaptic GABA receptor mediated responses which lead its antidepressant properties (Xinnong *et al*., 2012). Many studies have shown that barbiturates potentiate the GABA interneurons and induce intracellular responses that enhance neurogenesis and synaptic plasticity similar to what is seen in drugs that enhance antidepressant responses (Li *et al*., 2015). Studies have shown that the surge in GABA interneurons leads to the activation of the mammalian target of rapamycin complexes (mTOR) and other downstream intracellular pathways that promotes protein translation and neurogenesis (Li *et al*., 2015).

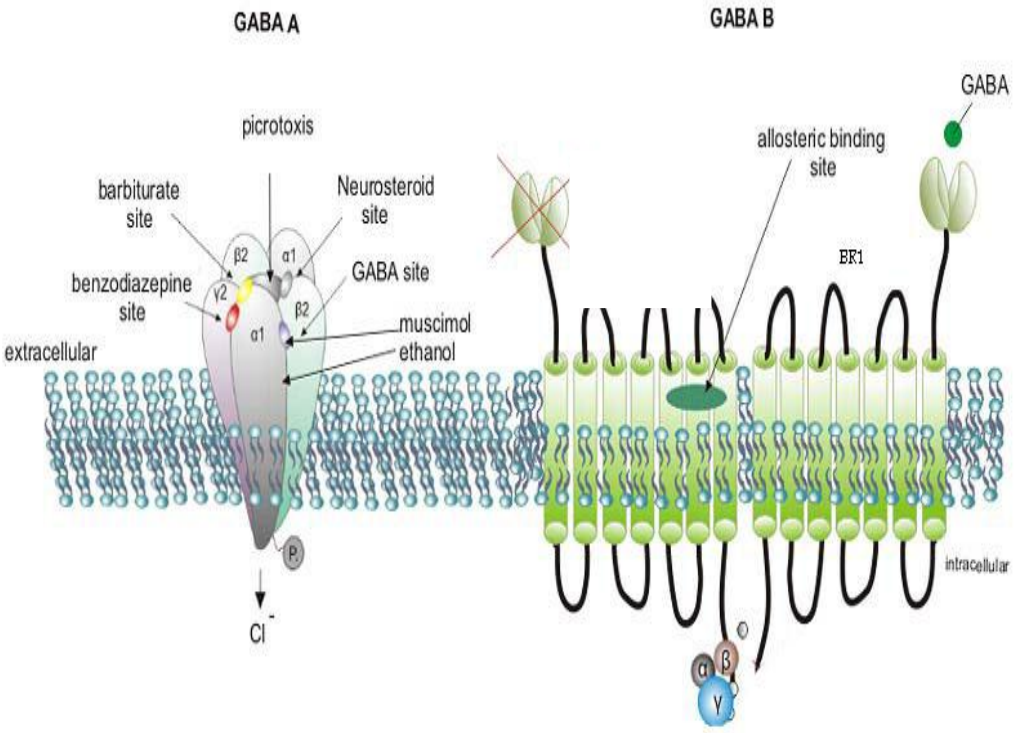

Figure 4: GABA receptors and their subtypes (Joanna *et al*., 2011).

CHAPTER THREE

3.0 Materials and Methods

3.1 Experimental Animals

A total of 108 Adult male Swiss Albino mice weighing between 18 to 22 grams were used for the study. The animals were sourced from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The animals were housed in cages containing dust-free sawdust bedding and were allowed free access to food and water *ad libitum*. All the experimental procedures were carried according to the use of animals in research, Ahmadu Bello University Research Policy, 2010.

3.2 Drugs/Chemicals

Phenobarbitone of analytical grade with NAFDAC registration number: 04-0074 was purchased from Vitabiotics (Nig.) Ltd, and was used throughout the experiment. Imipramine of analytical grade with NAFDAC registration number: A4-3899 was purchased from Rajat Pharmachem Ltd, Gujarat, INDIA and was used as the standard control. Distilled water served as the vehicle throughout the experimental phases. Sucrose pellets was obtained from Sigma Aldrich. Methylated spirit was used for cleaning site of injection.

3.3 Experimental Groupings

The study was conducted in two phases, comprising of an acute and a chronic phase respectively. In the acute phase of the study, the animals were divided into six groups of six animals per group for each of the neurobahavioural assays, i.e. the Tail suspension test (TST) and Forced swimming test (FST) respectively. Each mouse received a single intraperitoneal injection of the test drug (Phenobarbitone: 0.5 mg/kg, 2.5 mg/kg, 5 mg/kg and 10 mg/kg). Distilled water 10 ml/kg was used as the vehicle while Imipramine 20 mg/kg was used as the standard drug. In the chronic phase of the study, the animals were divided into six groups of six animals each, and were subjected to the chronic mild stress (CMS) model. At the end of the 30^{th} day, the CMS was terminated and the animals were further subjected to the Forced swimming test (FST), Sucrose preference test (SPT) and OFT (Open field).

3.4 Equipment/Apparatus

I. Forced swimming test apparatus

II. Tail suspension test apparatus

III. Weighing scale

IV. Syringes and needles (1ml, 5mls, 10mls)

V. Standard cages

VI. Methylated spirit and Cotton wool.

VII. Handkerchief

VIII. Stop watch

IX. Masking tape and Permanent markers

X. Mercury in glass thermometer

XI. Video camera

XII. Electric kettle

XIII. Measuring cylinder

3.4 Neurobehavioral Assessments

3.4.1 Acute depression study

3.4.1.1 *Forced swimming test (FST)*

The forced swimming test lasted for a period of two days and was carried out as a slight modification to the method of Porsolt *et al*. (1977). The FST involved the use of a rectangular glass cylinder of height 45cm and diameter 20cm and was filled with water to the 15cm mark (maintained at 21-23 ºC). On the first day, the animals were introduced into the glass cylinder and forced to swim for period of 15min until they became exhausted, then they were removed, dried and returned to their cages. On the second day, the animals were subjected to the same test 30 minutes after administration of the vehicle and the test drugs i.e. Phenobarbitone (0.5mg/kg - 10mg/kg), while (20mg/kg) Imipramine was used as the standard control, (10ml/kg) distilled water was used as the negative control. The animals were observed for a period of 6Min and the duration of time that the animal stayed immobile in the water i.e. without swimming or moving any of its limbs was taken as the immobility time.

3.4.1.2 *Tail suspension test (TST)*

Each mouse was suspended by an adhesive tape attached 1-2cm from the tip of their tail to a horizontal bar. The bar was raised 50cm from the floor, the mice were observed for a period of 6 minutes and the immobility time was recorded with the aid of a stop watch. At the end of the experiment the tape was removed and the mice were returned to their cages. Each animal was suspended 30 minutes after administration of the vehicle and the test drugs i.e. Phenobarbitone (0.5mg/kg -10mg/kg) while Imipramine (20mg/kg) as the standard control, (10ml/kg) distilled water was used as the negative control. The duration of time that the animal spent struggling was

taken as the mobility time. The immobility time was calculated as: Total exposure time - Mobility time.

3.4.2 Chronic depression study

3.4.2.1 *Chronic mild stress (CMS*)

The chronic mild stress method is used for inducing behavioural despair, anhedonia and hypo locomotion in experimental animals. The CMS method was carried out by subjecting the animals to a series of mild stressors for a period of 30 days. At the end of the 30th day, the animals were subjected to the forced swimming test, sucrose preference test and open field test respectively.

Prior to starting the experiment, the animals were trained to consume to 2% sucrose for 3 days in order to establish a baseline for their sucrose preference. Subsequently, the animals were then divided into six (6) groups of six (6) animals per cage based on their sucrose preference. Each animal received a single intraperitoneal injection of Phenobarbitone (0.5mg/kg-10mg/kg), while Imipramine (20mg/kg) was used as the positive control, (10ml/kg) distilled water was used as the negative control.

The CMS paradigms included 5hrs of food deprivation, 6hrs of water deprivation, cage tilting at 45º, cage soiling with 250mls of tap water for 6 hrs, and overnight illumination with bright light for 14 hours (Blanchard *et al*., 2001).

At the end of the 4th week, the CMS was terminated and the animals were fasted for a period of 14 hours and then exposed to 2% sucrose solution again (Willner, 2005).

3.4.2.2 *Sucrose preference test* (SPT)

The sucrose preference test was used to assay stress-induced anhedonia in experimental animals. Mice are trained to consume palatable solution containing 2% sucrose solution for about a week, and a base line for their sucrose preference was established. Subsequently, the mice were exposed to the CMS and their sucrose preference was being monitored weekly using a 50ml graduated cylinder. After terminating the CMS paradigms, the mice were fasted for period 14 hours and were subsequently exposed to the 2% sucrose solution. Their preference for palatable sucrose solution was taken as an indices of anhedonia which is a core sign of depression and was calculated using the formula $V= \pi.r^2.h$.

Where V= volume (in cm^3, which is equivalent to ml) and

$\pi \approx 3.1416$

h =distance between daily markings (in cm).

r = radius (half the interior diameter in cm) (Andrew *et al*., 2016).

3.4.2.3 *Open field test* (OFT)

The open field was made up of a white ply wood and Plexiglas measuring 42cm long × by 42cm wide and 30cm high. The floor of the apparatus was made with blue lines and divided into sixteen squares, with a central square measuring 18cm ×18cm. The animals were given Phenobarbitone (2.5mg/kg-10mg/kg), Imipramine (20mg/kg) and Distilled water (10ml/kg). Thirty minutes later, each animal was subjected to the open field test. Each mouse was placed individually at the left-hand corner of the apparatus and allowed to move freely for a period of 5 minutes. The number of line crossings and center crossings were recorded using a tally system. The line crossings were scored when the animal crosses one of the grid lines with all its four limbs. At the end of the 5^{th} minute, the animal is removed and the floor of the apparatus is cleaned with methylated spirit in order to avoid olfactory cues.

3.6 Statistical Analysis

Data obtained were analysed using SPSS version 20 and the results were expressed as Mean ± SEM. The data were analysed using one-way ANOVA followed by Dunnett's post-hoc test for multiple comparisons. Values with $p< 0.05$ were considered significant**.**

CHAPTER FOUR

4.0 Results

4.1 Acute Depression Study

4.1.1 Acute depression study: Tail Suspension Test (TST)

There was no statistical significant difference in the immobility time between the control group distilled water (10ml/kg) (216.5 ± 24.57) and Phenobarbitone treated groups 5 mg/kg, 2.5 mg/kg and 0.5 mg/kg with means (198.6 ± 26.4, 183.1 ± 21.2, 169.3 ± 25.5) respectively. However, at the dose of 10 mg/kg, Phenobarbitone significantly reduced the mean immobility time when compared to control (126.1±11.34*) (p<0.05). Similarly, Imipramine (20mg/kg) significantly reduced the immobility time when compared to control (60.1±7.6*) (p<0.05) as shown in figure: 4.1 below.

4.1.2 Acute depression study: Forced Swimming Test (FST)

There was no statistical significant difference in the immobility time between the control group, distilled water (10ml/kg) (75.6 6± 26.73) and Phenobarbitone treated groups 5mg/kg, 2.5mg/kg and 0.5mg/kg with means (54.33 ± 18.12, 64.0 ± 27.2, 32.16 ± 13.93) respectively. However, at the dose of (10 mg/kg), Phenobarbitone significantly reduced the mean immobility (5.5 ±1.70*) (p<0.05) when compared to control. Similarly, Imipramine (20 mg/kg) significantly reduced the immobility time when compared to control (60.1±7.6*) (p<0.05) as shown in Figure 4.2 below.

4.2 Chronic Depression Study

4.2.1 Chronic mild stress

4.2.1.1 *Chronic mild stress: forced swimming test*

There was no statistically significant difference in immobility time between the control group distilled water, (10ml/kg) (75.6 ± 26.7) and Phenobarbitone treated groups 5mg/kg, 2.5mg/kg and 0.5mg/kg with means (54.3 ± 18, 64.0 ± 27.2, 32.1 ± 13.9) respectively. However, at the 10 mg/kg Phenobarbitone treatment group there was a statistical significant difference in immobility time when compared to control (5.5±1.70*) (p<0.05). There was also a statistically significant

difference in immobility time between the standard control Imipramine 20 mg/kg (5.1 ± 1.35*) and the control group distilled water (10 ml/kg) as shown in figure 4.3 below.

4.2.1.2 *Chronic mild stress: Sucrose Preference Test* (SPT)

There was an increase in sucrose preference in the treatment groups when compared to control group 0.5 mg/kg, 2.5 mg/kg, 5 mg/kg (18.3 ± 1.54, 19.1 ± 1.24, 21.0 ± 1.59) respectively, though it was not statistically significant. But at the dose of (10ml/kg) (24.5 ± 1.06*) (p<0.05) Phenobarbitone significantly increased the sucrose preference when compared to control. There was a statistically significant increase in sucrose preference between the control group (17.5 ± 1.70) and the standard control Imipramine 20 mg/kg (24.6 ± 1.49*) (p<0.05) when compared to control as shown in figure: 4.4 below.

4.3 Open Filed Study

There was no statistically significant difference in line crossings between the control group distilled water (10ml/kg) (24.16 ± 1.90) and Phenobarbitone treated groups 10mg/kg, 5mg/kg, 2.5mg/kg, 0.5mg/kg with means (35.0 ± 5.76, 31.5 ± 2.36, 37.5 ± 4.60, 37.5 ± 4.65) respectively. There was no statistical difference between the control group (24.16 ± 1.90) and the standard control Imipramine 20mg/kg (38.3 ± 5.71) as shown in the table below 4.1 below.

Table 4.1: Effect of Phenobarbitone on exploratory behaviour of mice following Chronic Mild Stress

Treatment	**Line Crossings**
Distilled water (10ml/kg)	24.16 ± 1.90
PBT (0.5mg/kg)	37.5 ± 4.65 [ns]
PBT (2.5mg/kg)	37.5 ± 4.60 [ns]
PBT (5mg/kg)	31.5 ± 2.36 [ns]
PBT (10mg/kg)	35.0 ± 5.76 [ns]
Imipramine (20mg/kg)	38.3 ± 5.71 [ns]

[ns:] Not significant (compared with control)

Data represented as Mean ± SEM; Dunnet Post hoc test for multiple comparison; n=6

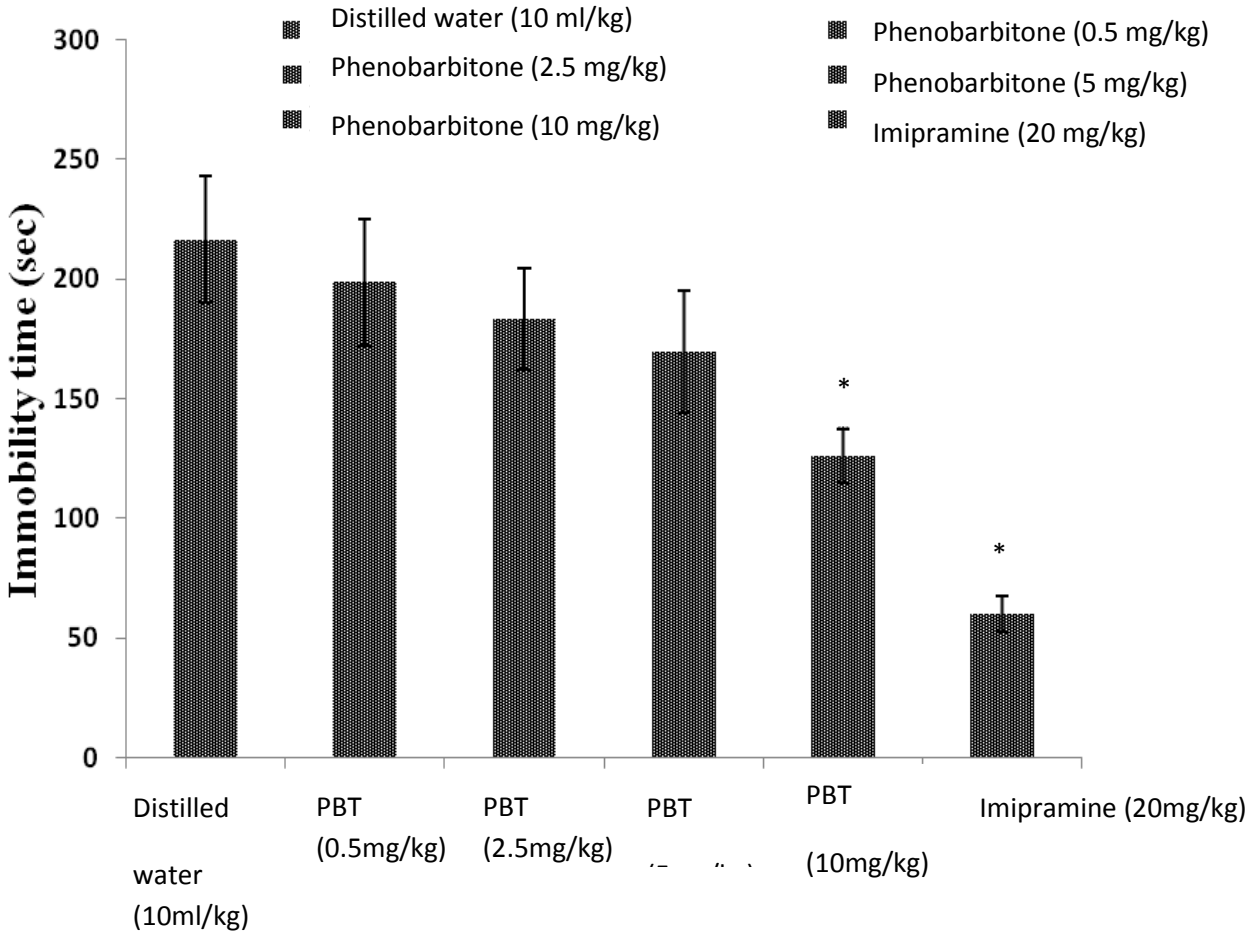

Figure 4.1: Effect of Acute Administration of Phenobarbitone on behaviour of mice in the Tail Suspension Test: Data presented as Mean ± SEM; $p<0.05$ (compared with control) PBT (Phenobarbitone); n = 6.

*Significance (p<0.05)

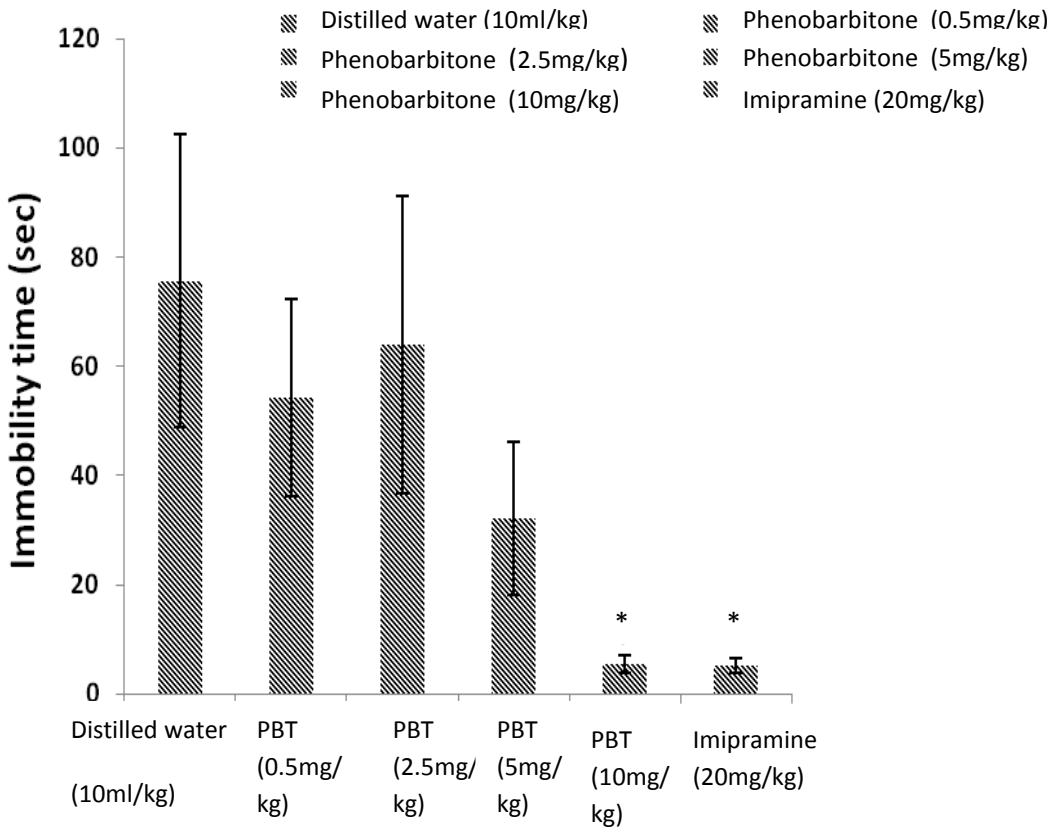

Figure 4.2: Effect of Acute Administration of Phenobarbitone on behaviour of mice in the Forced Swimming Test: Data presented as Mean ± SEM; $p<0.05$ (compared with control) PBT (Phenobarbitone); n = 6.

*Significance ($p<0.05$)

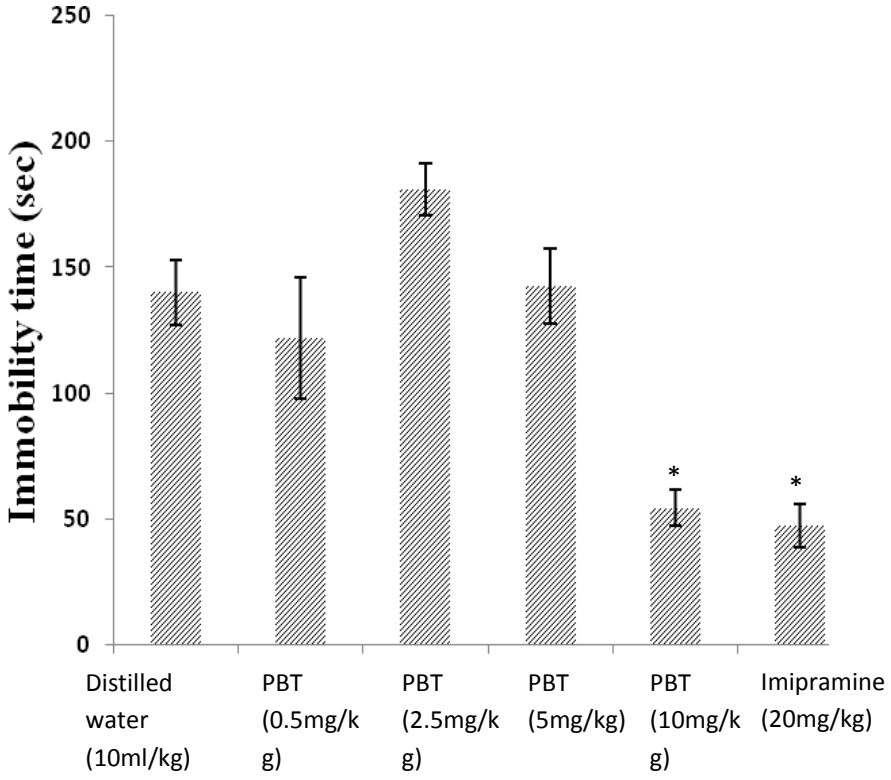

Figure 4.3: Effect of Chronic Administration of Phenobarbitone on behaviour of mice in the Forced Swimming Test: Data presented as Mean ± SEM; $p<0.05$ (compared with control) PBT (Phenobarbitone); n = 6

*Significance (p<0.05)

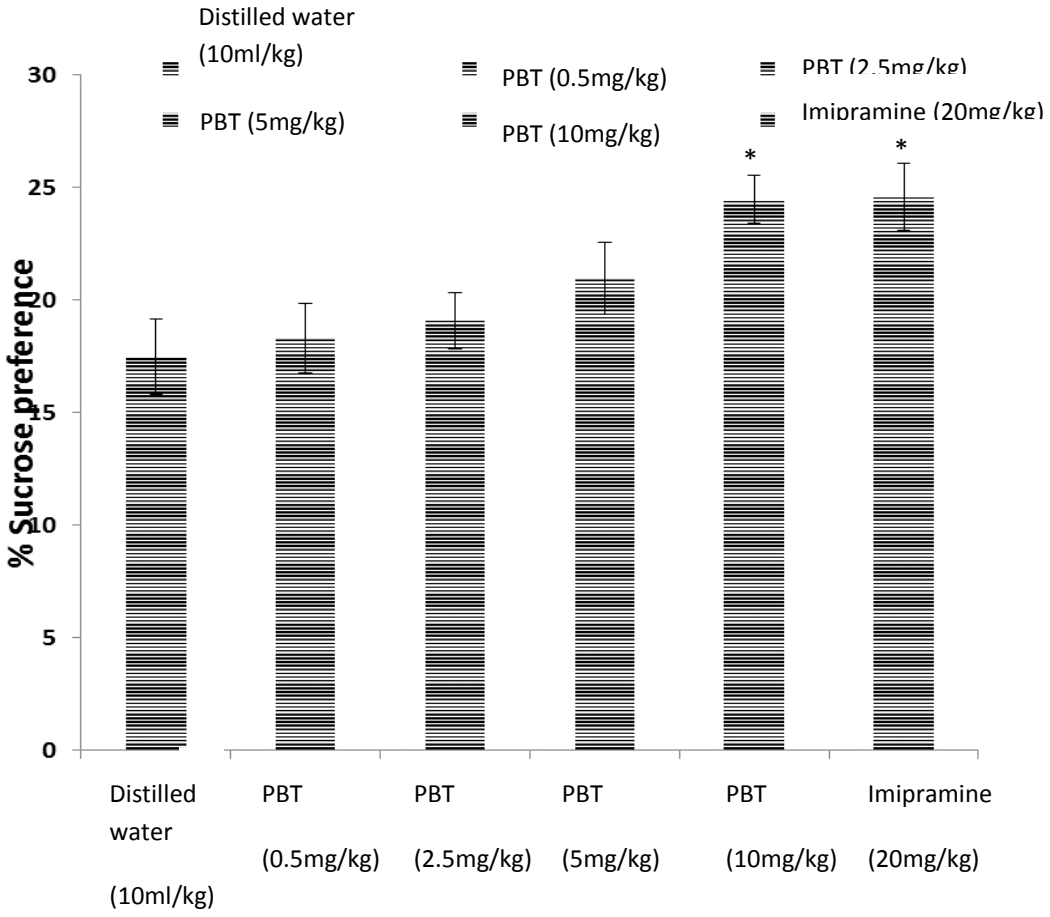

Figure 4.4: Effect of Chronic Administration of Phenobarbitone on behaviour of mice in the Sucrose Preference Test: Data presented as Mean ± SEM; $p<0.05$ (compared with control) PBT (Phenobarbitone); n = 6

CHAPTER FIVE

5.0 Discussion

This study attempted to provide some scientific basis on the antidepressant properties of the GABAergic system. The study used stress as a tool to induce CNS depression in the experimental animals and Phenobarbitone showed antidepressant potential. Studies have shown that stress is a strong epigenetic that alters the way cells respond and interpret information from the DNA, this ultimately leads to the formation faulty proteins and hence synaptogenesis; especially synaptic plasticity and long term potentiation (Amy *et al*., 2016).

In the acute phase of the study, result obtained in the TST showed a statistical significant difference in immobility at the highest dose tested (10mg/kg) treatment group ($p<0.05$) when compared to control, but there was no statistically significant difference when between the treatment groups, even though a dose dependent decrease in immobility time was observed between the groups. This is in line with the work of Nagatani *et al*., (1987) which showed that agonists of the GABA-benzodiazepine receptor complex decreased immobility time in experimental animals in a dose dependent manner. This finding could also be attributed to the less aversive nature of the TST, which assays behavioural despair in mice as a core symptom of depression in mice models of depression (Adem *et al*., 2012).

In the FST group of the acute study, a statistically significant difference in immobility time was observed at the (10mg/kg) group when compared to control group ($p<0.05$). But there was no statistically significant difference when compared to other treatment groups. This is also in line with the work of Abdallah *et al*., (2014), which showed that drugs that enhance central GABA activity such as Imipramine, Diazepam, Vigabatrin and Alprazolam possess antidepressant potentials by reducing the immobility time in the FST. But the result did not show a dose dependent decrease in immobility time in the treatment groups when compared to control as observed in the TST. This could be attributed to stress- induced hypothermia seen in experimental animals when using the FST (Willner and Mitchell, 2002).

But at the (2.5mg/kg) group there was an increase in immobility time when compared to the control and other treatment groups though it was not statistically significant, and this could be due to the false positive or false negative results obtained when using the forced swimming test in laboratory animals due to the stressful nature of the procedure and aversive nature to water displayed by most rodents (Vincent *et al*., 2009).

In the chronic phase of the experiment, results obtained from the FST showed a statistically significant difference in immobility time at the (10 mg/kg) group when compared to the control group (10ml/kg) distilled water ($p<0.05$), this indicates an antidepressant activity at dose of (10mg/kg). But at the dose 2mg/kg there was an increase in the immobility time when compared to other treatment groups, and this can be attributed to the poor face validity of the forced

swimming test compared to other methods of screening antidepressant properties in laboratory animals (Yang-Hee *et al*., 2014).

In the SPT, a statistically significant difference in sucrose preference was seen at the (10mg/kg) group when compared to control group (10ml/kg) distilled water; (p<0.05). This indicates anhedonic behaviour at this dose which is a core sign of depression (Jean-Luc, 2002). The increase in sucrose preference observed at this dose indicates antidepressant activity. Studies have shown that mice exposed to the CMS paradigms show a significant decrease in sucrose preference compared to the control group (Willner, 1987). Whilst agents that possess antidepressant properties increases sucrose preference in mice subjected to the CMS paradigms. This result is in line with the work of (Willner, 2005; Schweizer, 2009) which indicate that an increase in sucrose preference after exposure to CMS is a sign of antidepressant behaviours in laboratory animals. The result also showed a dose dependent increase in sucrose preference across the treatment groups (0.5mg/kg- 5mg/kg) when compared to control. This further affirms that the result obtained from both phases of the study; that at (10mg/kg) Phenobarbitone possess antidepressant activities.

In order to rule out the role of locomotory activity in the study, the animals were subjected to the open field test. The OFT has been widely used to evaluate locomotory activities in experimental animals and also to screen the autonomic effects of drugs (Kathleen, 2009). Results from this study in the OFT did not show any increase in line crossings throughout the treatment groups when compared to control. Therefore, all the effects observed in the TST, FST and CMS were due to antidepressant properties and not due to an increase in stimulant activity.

The possible mechanism by which phenobarbitone possesses antidepressant property in the TST and FST could be via enhancing central GABAergic activity, as shown in the work of Abdalla *et al*. (2014). This could be achieved by enhancing the GABAergic receptors such as $GABA_A$ and $GABA_B$ receptors respectively (Möhler, 2012). Another possible explanation for antidepressant property of phenobarbitone observed in this study could be due to the effect GABAergic inputs on monoaminergic pathways in the raphe nucleus by GABA (Maejima *et al*., 2013). GABAergic inputs have been shown to enhance the central monoaminergic pathways in the brain by increasing the expression of the monoaminergic receptors such as α1 α2 adrenoceptors (Elhwuegi, 2004). Furthermore, GABA interneurons are known to be central in the rapid antidepressant responses of drugs that alter gabergic signalling such as ketamine and lithium (Abelaira *et al*., 2014). Thus alteration in gabergic signalling is central in the antidepressant properties of drugs that act via the Benzodiazepine-GABA receptor complex (Anita *et al*., 2012) and could be the possible mechanism by which phenobarbitone exerted its antidepressant properties.

CHAPTER SIX

6.0 Conclusion

From the study above, Phenobarbitone showed a statistical significant difference decrease in mean immobility at the highest (10 mg/kg) treatment group in both the acute and chronic phase of the study, using neurobahavioural assays in the FST, TST, CMS and SPT. But there was no statistical significant increase in mean immobility time at the lower dose tested in the 5mg/kg, 2.5mg/kg and 0.5 mg/kg treatment groups treatment groups for both phases of the experiment when compared to the control. Also, there was a reversal in hedonic tendency induced by chronic mild stress indicated by an increase in sucrose preference in both phases of the study and was statistically significant when compared to control at the 10 mg/kg treatment group, this indicates antidepressant activity at this dose. On exposure to the open field test, the mice did not show a statistical significant increase in line crossings in all the treatment groups compared to control, which indicates that the effect observed in the both phases of the study was due to antidepressant activity and not due to increase in locomotory activities or stimulant effect of the test drug.

6.1 Recommendations

Based on the findings in this study, the following recommendations are made:

I. The need to carry out biochemical assays on the possible role of intracellular proteins and neurotrophic proteins such as BDNF and mTOR complexes on the antidepressant property of phenobarbitone.

II. Phenobarbitone at the dose of (10mg/kg) significantly possesses antidepressant property as evidenced by a decrease in immobility time in the FST and TST.

III. Phenobarbitone possesses hedonic activity by significant increase in sucrose consumption following chronic mild stress.

6.3 Contributions to Knowledge

I. The study showed that phenobarbitone at a dose of 10 mg/kg possesses significant antidepressant activity by reducing the mean immobility time in both phases of the study

(Acute Phase: TST126.1±11.34*, FST54.5±7.34*; Chronic Phase: FST54.5±7.34*) when compared to control (p<0.05).

II. The study showed that Phenobarbitone possesses hedonic activity by increasing sucrose preference following exposure to Chronic mild stress in all the treatment groups (21.0 ± 1.59, 19.1 ± 1.24, 18.3 ± 1.54, 24.5 ± 1.06) when compared to control.

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Appendices

APPENDIX I

Table 4.1: Effect of Acute administration of Phenobarbitone on CNS Depressant-like Behaviors in Mice using the Tail Suspension Test n = 6

Treatment	Period of Immobility (s)
Distilled water (10ml/kg)	216.5 ± 26.5
PBT (0.5mg/kg)	198.6 ± 26.4 [ns]
PBT (2.5mg/kg)	183.1 ± 21.2 [ns]
PBT (5mg/kg)	169.3 ± 25.5 [ns]
PBT (10mg/kg)	126.1 ± 11.34*
Imipramine (20mg/kg)	60.1 ± 7.6*

The mean difference was statistically significant at *p<0.05*

[ns] Not statistically significant

APPENDIX II

Table 4.2: Effect of Acute administration of Phenobarbitone on CNS Depressant-like Behaviors in Mice using the Forced Swimming Test n = 6

Treatment	Period of Immobility (s)
Distilled water (10ml/kg)	75.66 ± 26.73
PBT (0.5mg/kg)	54.33 ± 18.12 [ns]
PBT (2.5mg/kg)	64.00 ± 27.29 [ns]
PBT (5mg/kg)	32.16 ± 13.93
PBT (10mg/kg)	5.5 ± 1.70*
Imipramine (20mg/kg)	60.1 ± 7.6*

The mean difference was statistically significant at *p<0.05*

[ns] Not statistically significant

APPENDIX III

Table 4.3: Effect of Chronic Administration of Phenobarbitone on CNS Depressant-like Behaviors in Mice using the Forced Swimming Test n = 6

Treatment	Period of Immobility
Distilled water (10ml/kg)	75.6 ± 26.7
PBT (0.5mg/kg)	54.3 ± 18.1n^{ns}
PBT (2.5mg/kg)	64.0 ± 27.2^{ns}
PBT (5mg/kg)	32.1 ± 13.9^{ns}
PBT (10mg/kg)	5.5 ± 1.70*
Imipramine (20mg/kg)	5.1 ± 1.35*

The mean difference was statistically significant at *p<0.05*

^{ns} Not statistically significant

APPENDIX IV

Table 4.3: Effect of chronic administration of Phenobarbitone on CNS Depressant-like Behaviors in Mice using the Sucrose Preference Test n = 6

Treatment	Sucrose Preference

Distilled water (10ml/kg)	17.5 ± 1.70
PBT (0.5mg/kg)	18.3 ± 1.54[ns]
PBT (2.5mg/kg)	19.1 ± 1.24[ns]
PBT (5mg/kg)	21.0 ± 1.59[ns]
PBT (10mg/kg)	24.5 ± 1.06*
Imipramine (20mg/kg)	24.6 ± 1.49*

The mean difference was statistically significant at *p<0.05*

[ns] Not statistically significant

APPENDIX V

Table 4.4: Effect of chronic administration of Phenobarbitone on Line crossings using the Open Field Test n = 6

Treatment	**Line Crossings**
Distilled water (10ml/kg)	24.16 ± 1.90
PBT (0.5mg/kg)	37.5 ± 4.65 [ns]

PBT (2.5mg/kg)	37.5 ± 4.60 [ns]
PBT (5mg/kg)	31.5 ± 2.36 [ns]
PBT (10mg/kg)	35.0 ± 5.76 [ns]
Imipramine (20mg/kg)	38.3 ± 5.71 [ns]

[7uns] Not statistically significant