THE ROLE OF MONOAMINERGIC SYSTEMS IN ANXIOLYTIC AND ANTI-DEPRESSANT POTENTIALS OF METHANOL EXTRACT OF *ARTOCARPUS ALTILIS* FOSBERG IN LABORATORY MICE

BY

AJAH, AUSTIN. AZUBUIKE (M.Sc.)

Matric number: 153936

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ABSTRACT

Anxiety is described as a threat to psychological, physiological and behavioral state, while depression is a potentially life-threatening disorder that presents with decreased mood.Folkloric use of *Artocarpus altilis* includes alleviation of mood swings. Nevertheless, there is limited information on the scientific evaluation of the effects of the plant on anxiety and depression. Therefore, this study was designed to investigate the anxiolytic and antidepressant potentials of *Artocarpus altilis* in laboratory mice and the role of monoaminergic systems.

Artocarpus altilis was obtained from the Botanical Garden, University of Ibadan, authenticated at FRIN (FHI No.: 110483), shade-dried, pulverised and macerated in methanol for 72 hours to obtain Methanol Extract of *Artocarpus altilis* (MEAA). One hundred and eight male Swiss mice (18-25 g) were used in three studies (I, II and III). Study I had 5 groups (n=6) for anxiolytic and antidepressant studies. The anxiolytic effects of MEAA (50, 100 and 200 mg/kg, p.o.) were investigated using the Elevated Plus Maze (EPM), Elevated Zero Maze (EZM) and Light and Dark Test (LDT); while its antidepressant effects were evaluated using Forced Swimming Test (FST) and Tail Suspension Test (TST). Diazepam (1 mg/kg, i.p.) was used as the reference anxiolytic drug, imipramine (10 mg/kg p.o.) served as the reference antidepressant drug, while controls received normal saline. Study II had 4 groups (n=6); Imipramine, MEAA 50, 100 and 200 mg/kg, respectively, in which Lipopolysaccharide (0.5 mg/kg, i.p.) was used to induce depression. Reduced glutathione, Superoxide Dismutase (SOD), Nitric Oxide (NO) and Thiobarbituric Acid Reactive Substances (TBARS) were determined in brain tissue by standard methods using spectrophotometry. In study III, 4 groups (n=6) were used to test various mechanisms; Group 1 (200 mg/kg MEAA, 200-MEAA), Groups 2-4 were pre-treated with metergoline (5-HT receptor antagonist, 4 mg/kg, i.p.), prazosine (α_1 adrenoceptor antagonist, 1 mg/kg, i.p.) and sulpiride (D_2 receptor antagonist, 50 mg/kg, i.p), respectively before 200 mg/kg MEAA and TST. Data were subjected to descriptive statistics and analysed using ANOVA at $\alpha_{0.05}$.

The 200-MEAA showed significant increase in time spent in the open arms of EPM (63.0±11.0 s), EZM (74.5±10.2 s) and light chamber in LDT (123.3±1.2 s) relative to control (21.5±4.4; 53.7±0.3; 51.4±4.8 s). There was also a significant increase in the number of entries into the open arms of EPM (5.0±0.4), EZM (10.3±0.3) and LDT (5.0±0.4) relative to

control (2.5±0.3; 5.3±0.9; 4.8±0.4). There was a significant decrease in immobility time in 200-MEAA in FST (28.8±4.9 s) and TST (61.3±7.5 s) relative to control (174±15.9; 174.2±6.9 s). Lipopolysaccharide+200-MEAA decreased immobility time in FST (38.2±11.1 s) relative to control (190.0±7.9 s). There were increases in the levels of TBARS (2.0±0.2 μmolMDA/g), SOD (24.6±0.9 ng/ml) and NO (144.9±7.3 μM/mg) in 200-MEAA relative to control (1.2±0.2 μmolMDA/g; 18.6±0.9 ng/ml and 128±5.8 μM/mg, respectively). In study III, there was a significant decrease in immobility time in 200-MEAA (170.3±7.7 s) when compared with metergoline, prazosine and sulpiride (200.5±18.5, 175.2±16.5, 184.3±11.3 s, respectively).

Methanol extract of *Artocapus altilis* demonstrated antidepressant and anxiolytic potentials. These effects are mediated through serotonergic, noradrenergic and dopaminergic receptors.
Keywords: Artocarpus altilis,Antidepressant potential, Anxiolytic activity, Monoaminergic systems, lipopolysaccharide
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DEDICATION

This piece of work is dedicated to God almighty, the author and finisher of my faith.

My Lovely wife, Mrs Grace Austin-Ajah, and My late father, Mr Ajah, Cletus Ofuru, who sacrificed his comfort to make me the man I am today,

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Finally, to my wife and Best friend, my heart Mrs. Grace Austin-Ajah, you mean the world to me. I LOVE YOU TO THE MOON AND BACK.

CERTIFICATION

I certify That This Work titled '**The role of monoaminergic systems in anxiolytic and anti-depressant potentials of methanol extract of *Artocarpus altilis* Fosberg in laboratory mice**' was carried out by Mr. Austin Azubuike,**AJAH** in the Department of Physiology, College of Medicine, University of Ibadan.

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Supervisor

Dr. S. A. Onasanwo

B.Sc. Physiology (Ibadan), M.Sc.Physiology (Ibadan), Ph.D. Physiology (Ibadan)

Department of Physiology

College of Medicine

University of Ibadan, Nigeria.

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CHAPTER ONE

1.1 BACGROUND OF THE STUDY

The innate ability to respond to stimuli (irritability) arising from both internal and external environments is considered one of the basic characteristics of living things. These stimuli could be pleasurable or not, hence the response of an organism to various stimuli differs. Psychologists regard aversive stimuli as hostile stimuli that cause alterations of behaviour. In the course of punishment, when an aversivestimulus is applied instantly after an action, the probability of the action to occur in future is reduced (Jones and McCaughey, 1992).

An aversive stimulus is an obnoxious event geared towards decreasing the likelihood of behaviour after the situation is given as an outcome (i.e., penalty). Though an aversive inducement might similarly intensify the possibilityof behaviour when it is detachedas a consequence, and in this way, it will operateas negative reinforcement (Jones and McCaughey, 1992).An aversive stimulus is explainedin learning perspectivesto consist ofstimuli, when used as a consequence to get a response (Lerman and Vorndran, 2002).

Anxiety-related ailments and depression are amongst the most expensive categories of mental health disorders, both in ill health as well as economic expense (DiLuca and Olesen, 2014; Baldwin *et. al*., 2014). Occurrence of anti-anxiety and anti-depressant medications remained one of the main attention of the pharmacological productiveness as well as intellectual neuropsychiatric investigations, however not any novel medication categories have stood to be approved in the meantime than the presentation of selective serotonin-reuptake inhibitors (SSRIs) in addition to more anti-depressants to aid the cure for these conditions (Griebel and Holmes, 2013).

Anxiety is generally explained as a physiologic, psychological and behavioural condition stimulated within organisms through danger towards its welfare and/or existence, be it real or

perceived (Steimer, 2002).Fear is interrelated with anxiety, and can be expressed as a reaction of an organism to existent or perceived dangers that might alter its homeostasis. These reactions might involve physiologic (heart beat rate elevation alsopressure of the blood etc.), behaviour related parameters like inhibition of current actions, visualising and avoiding the location of the risk. Once this reaction is extreme or unbearable, it implies pathologic anxiety (DSM-IV, 1994).

Depression is a potential fatal condition affecting several millions of persons globallyoccurring at any age, from infancy to later in lifetime (Alexander *et. al.*, 2002). Major depression is most likely to affect people between the ages of 45 and 65 as people in middle age are at the top of the bell curve for depression, but the people at each end of the curve, the very young and very old, may be at higher risk for severe depression (Alexander *et. al.*, 2002). This disorder presents with serious suffering and interruption of lifespan, if not properly managed, could be deadly. This psychopathological state requires a triad of indications with anhedonia, low energy or fatigue, and low mood. It may also present with symptoms like guilt, poor self-confidence, sleep as well as psychomotor disturbances, suicidal predispositions, gastrointestinal and autonomic disturbances. It is rather a complicated episode that has several subdivisions, and possibly more than a single etiology (Alexander *et. al.*, 2002).

Fruits represent an abundant source of nutrients and non-nutritive bioactive compounds which are mostly associated with reduced risk of many non-communicable/chronic diseases (Wolfe *et al*., 2008). *Artocarpus altilis* (Bread Fruit) belongs to the family of Moraceae; made up of about 60 types, natural to the south-east Asia, Indian subcontinent and Australia (Jarrett, 1959; Kochummen and Fuster, 2000; Forsberg, 1939; Rousseau 1955).

1.2 STATEMENT OF THE PROBLEM

Anxiety and depression has been projected to affect millions of lives all over the world thereby resulting in the loss of the lives of loved ones and bread winners inclusive. Although, there are a lot of anxiolytic and antidepressant drugs, there has been need for more effective and efficacious anxiolytic and antidepressant drugs with fewer side effects.

Extracts of latex, sap, leaves, stem and bark of *Artocarpus altilis*has been reported to be effective in the treatment of broken bones, skin ailments and fungus infection such as thrush, ear infection, sore eyes and headache among others (Ragone, 2006). However, significant research on extracts of the fruits has not been done. Also, no significant study has been reported on the effect of the fruit extracts *of Artocarpus altilis* on anxiety and depression.

1.3 JUSTIFICATION OF THE STUDY

Anxiety related disorders and depression affect about 350,000,000 persons. World Mental Health Review organized around Seventeen nations discovered that on average approximately one in twenty persons testified of experiencing an incident of depression (Alonso *et. al.,* 2011). An anxiety disorder is estimated to be about 30% of mood conditions (Young *et al.,* 2004). Statistically, 47.5% of patients with major depressive disorders also experience disorders related to anxiety, where 26.1 percent of patients suffering from anxiety disorders similarly experience Major Depressive Disorder, (MDD). However, nearly eight percent of patients seeking medication have Generalized Anxiety Disorder, (GAD) (Beekman *et al.,* 2000). Depression has been estimated to be the second prevalent condition after heartdisease by the year 2020(Alonso *et. al.,* 2011).

Medicinal usage of plants and plant productsaimed at research work is by no means new in practice but is as old as mankind and herbs are still the center ofmodern day drug discovery (Antes and Erzdogral, 2003). Fruits represent an abundant source of nutrients and non-nutritive bioactive compounds which are mostly associated with reduced risk of many non-communicable/chronic diseases (Wolfe *et al*., 2008).

Artocarpus altilis (Bread fruit) is wildly consumed by the people of southern Nigeria amongst other parts of the globe in different delicacies. This fruit is well revered due to its exotic nature and many healing properties have been ascribed to its efficacy in the treatment of broken bones, headache to skin ailments and fungus infection such as thrush, ear infection and sore eyes (Ragone, 2006).

Its natural abundance of numerous bioactive compounds of which some are neuroactive like arylbenzofurons (Shirajum *et. al,* 2015) have not been extensively examined for neurobehaviour and general CNS activities. Considering the analgesic and CNS depressantaction of *Artocarpus heterophyllus* seed of methanolic extract (Shirajum *et. al,* 2015), close specie to *Artocarpus altilis*, it is conceivable that, Methanol Extract of *Artocarpus altilis*(MEAA) may possess some effects on certain neurobehaviours. Hence, the needs to assess the impacts of *Artocarpus altilis* seed extract on anxiety anddepression in mice and the possible role of monoaminergic systems.

1.4 AIM OF THE STUDY

To accessthe anxiolytic and anti-depressant potentials of methanol extract of *artocarpus altilis* fosberg and the possible role of monoaminergic systems in laboratory mice**.**

1.5 OBJECTIVES OF THE STUDY

- To access the effect of MEAA on anxiety-like behaviour using elevated plus maze, elevated zero maze, light/dark board tests.
- To access the effect of MEAA on depression-like behaviour using forced swimming test and tail suspension tests.
- To examine the effect of MEAA on locomotor activity in open field model.
- To examine the effect MEAA on biochemical parameters in LPS-induced depression-like behaviours.
- To elucidate the possible mechanism(s) through which MEAA exerts its anti-depressant activity.

CHAPTER TWO

2.0 REVIEW OF LITERATURE

2.1 ANNALS OF ANTI-DEPRESSANTS

Curative methodologies to emotional ailments date back to 1950, through presentation of imipramine and iproniazid. Preceding medical induction of the first set of antidepressants, the curative instruments engaged into handling temperament ailments remained very uncommon (Judd, 1998). In the early 20th century the agents used were chloral hydrate, barbiturates, amphetamines, and even opiate derivatives in agitated melancholic patients. During the first half of that century, and excluding biological treatments (insulin comas, chemical and electrical shock therapy, or the famous “sleep cures”), whose use was widespread, the only chemical preparations available to physicians were some non-specific ones, such as succinic dinitrite, malonic nitrite or lactic acid, whose antidepressant results were rather unsatisfactory (Sandler, 1990).

The introduction of the so-called atypical, heterocyclic or “second generation” antidepressants (maprotiline, nomifensine, trazodone, mianserine, among others) in the 1970s did not bring the heralded momentous progress, from either the therapeutic or safety perspective, except in individual cases, with respect to the classical antidepressants (Sandler, 1990). Conversely, the medical presentation of selective serotonin reuptake inhibitors (SSRIs), later, within 1980, again, transformed treatments like introductory to new families of antidepressants (Sandler, 1990). However, all the drugs maintain a common pathway in their mechanism of action as classical drug, namely, alteration in mono-aminergic neurotransmission at synaptic levels.

2.2 THE ROLE OF MONO-AMINERGIC SYSTEM ON DEPRESSION

A key biological and chemical hypothesis of depression remains the monoaminergic hypothesis. It proposes that depression is triggered by physiological insufficiency in the amount of monoamines (Noradrenalin, dopamine and serotonin) in the brain. Decreased amount of monoamines is believed to be the major factor that triggers depressive condition. For example, decreased monoaminergic metabolite level was indicated in Cerebrospinal fluid of depressed patients(Gold *et al*, 1998).

2.2.1 MONOAMINERGIC THEORIES OF DEPRESSION

The 1960s saw the flourishing of mono-aminergic theories of depression, which postulated a functional deficiency of noradrenergic or serotonergic neurotransmission in certain brain areas as a primary cause of these pathologies (Coppen, 1967).

i. Noradrenergic hypothesis (Catecholaminergic Hypothesis)

Catecholaminergic hypotheses, the initial postulation, was established upon the earlier statements concerning the consequences of the anti-depressants newly discovered; the hindering of reuptake of noradrenaline at synaptic level by imipramine (Glowinski and Axelrod, 1964), and the fact that reserpine, an alkaloid that produces an emptying of noradrenaline in nerve endings, caused depressive symptoms in a high percentage of patients on being used as an antihypertensive (Godwin *et. al*., 1971). This hypothesis on the biological mechanism of depression, presented by Joseph J. Schildkraut of the Massachusetts Mental Health Center (Boston) in a classic work published in 1965, suggested that this pathology was due to a fall in noradrenaline level in the intersynaptic cleft: “some depressions, if not all, are associated with an absolute or relative deficit of catechol amines, particularly noradrenaline, in important adrenergic receptors in the brain. Contrariwise, elation may be associated with an excess of such amines” (Schildkraut, 1965).This so-called “noradrenergic

hypothesis of depression" set off an avalanche of studies on the role of the noradrenergic system in the genesis of affective and other psychiatric disorders.

ii Serotonergic Hypothesis

Since 1952, thanks to the work of Betty Twarog, a researcher in Professor John Welsh's laboratory at Harvard, it had been known that serotonin was a brain neurotransmitter (Twarog, 1988). A group led by Alec J. Coppen of the Neuropsychiatric Research Institute, which belonged to the Medical Research Council of London demonstrated that the administration of tryptophan, a precursor of serotonin, to depressed animals boosted the therapeutic effects of MAOIs (Coppen *et al*, 1963). Another group led by Dutch psychiatrist Herman M. Van Praag of the Department of Biological Psychiatry at Groningen University, working initially with the biochemist Bart Leijnse, concluded that there were reasons to acknowledge a relationship between MAO inhibition and antidepressant action, and between serotonergic dysfunctions and the appearance of certain types of depression (Van Praag, 2007).However, this serotonergic hypothesis was postulated without a clear demonstration of neuro biochemical correlates at a central level, but rather on the basis of studies of variables related to peripheral serotonergic dysfunction, basically at platelet level (Van Praag, 2007). The definitive extrapolation of these hypotheses to CNS functioning did not take place until the introduction of more modern techniques. Thus, in 1968, Arvid Carlsson and colleagues at the University of Gothenburg, Sweden, described for the first time how TCAs blocked the reuptake of serotonin at a brain level (Carlsson *et al*, 1968), leading to the postulateion of serotonergic theory of depression, as opposed to the catecholaminergic hypothesis, based on a deficit of serotonin at an inter-synaptic level in certain brain regions (Lapin and Oxenkrug, 1969). However, it was confirmed that TCAs and electroconvulsive therapy improved the efficiency of serotonergic transmission either through sensitization of post-synaptic receptors

or desensitization of pre-synaptic receptors, which usually reduce the release of serotonin in the synaptic cleft or inhibit the frequency of discharge of serotonergic neurons (Blier and De-Montigny, 1994). With all of these experimental observations it could also be concluded that a fall in synaptic levels of serotonin, in certain brain areas, was one of the biochemical causes of depressive disorders.

iii. Dopaminergic (DA) hypothesis of depression

It wasn't until the mid-1970s that a role for dopamine (DA) in depression was postulated (Randrup *et al*, 1975). The primary reason for the limited focus on DA was the finding that the efficacy of tricyclic antidepressants (TCAs) stemmed from their ability to inhibit the reuptake of NE and/or 5HT. However, a long-standing conundrum associated with the original monoamine hypothesis is that the reuptake inhibiting effects of TCAs (and SSRIs and SNRIs) occur within hours of drug ingestion, but their antidepressant effects take longer time to occur (Schildkraut, 1965). Despite this theoretical underpinning, research on the role of DA in depression has been largely overshadowed by research on noradrenaline (NA)-and serotonin (5HT). Recent findings clearly warrant scrutiny of the role of DA in the pathophysiology of depression and, moreover, whether there exists a "dopaminergic dysfunction" subtype, characterized by a poor response to antidepressants that act primarily on 5HT or NE neurons. There is now an emerging consensus that the majority of depressed patients treated with selective serotonin reuptake inhibitors (SSRIs) and selective serotonin/norepinephrine reuptake inhibitors (SNRIs) do not attain remission, this is due, in part, to the lack of effects of SSRIs and SNRIs on DA neurons (American Psychiatric Association, 2000).

2.3 ANIMAL MODELS OF DEPRESSION

Animal models remain usually perceived to be an effort towards replication of human's health conditions in a research animal laboratory (Sarter and Bruno, 2002). Though symptoms of psychiatric conditions remain often or frequently revised in addition to the pathogenesis been revisited (Sarter and Bruno, 2002), several restraints are required prior to declaring or exploiting animal model of depression.

There are quite many models of psychopathology relevant to mood disorders (Nestlers *et al*, 2002). Most of them are models for drug screening which has remained imperfect approximation of the target pathology (Willner, 1990). The important aim has always been to find models which would allow direct penetration into disease mechanisms and underlying pathological processes in the brain. The behavioural phenomenon used in the animal model has been validated mainly with pharmacological means; they react reliably to antidepressant pharmacotherapy (Willner, 1990).

2.3.1 MAJOR EXPERIMENTAL MODELS OF DEPRESSION IN ANIMALS (RODENTS)

i. Model of behavioural despair

The behavioural despair model refers to rats/mice forced swimming test (FST), and mice tail suspension test (TST). Easy, rapid, in addition to responsive model of animal experimentation is utilized in trial assessment of antidepressants. FST is usually a recognized animal model of depression for appraising the antidepressant properties (Nestlers *et al.*, 2002).

ii. Model of learned helplessness

The model of learned helplessness is a condition wherein an animal that has learned to behave helplessly when it is repeatedly subjected to a stress stimulus (e.g., electric shock) from which it cannot escape. Eventually, more behaviour deficits incur in the subsequent cognitive tasks. The model, highly sensitive to anti-depressants, can be employed for medicine screening and the study of their effects.Learned helplessness and FST utilize the decline inmovement following traumatic exposure (Sherman *et. al.,*1982). Animalsundergoing learned helplessness demonstrate DA exhaustion inside nucleus accumbens as well as caudate nucleus can stay blocked through pre-treatment by DA agonists (Anisman*et al*., 1979). Anxiety level is a strong influence on risk assessment in the sense that anxiety will make an animal to perceive a safe circumstance as very risky or even as life threatening thereby making the animal to avoid or move away from such a situation or location. This is the rationale behind learned anxiety test. (Geller, 1979)

2.3.2 RESEARCH ON DEPRESSION USING THE RODENT (RATS AND MICE); FORCED SWIMING TEST

In 2013, Piotrowska *et al,*studied the participation of monoamine system following action that suggests an antidepressant function of chromium chloride when subjected to forceswimming test depression model on rats as well as mice using Porsolt and co-workers' method (Porsolt *et al*,1978).

Animals were kept within a cylinder made up of 40 centimeterin height, and diameter of 20 centimeter, enclosing 30 centimeter water, conserved with a temperature of 25°c, for 6 minutes (Porsolt *et al*,1977). Immobility (period of continued passive floatation by the animal in the water) was determined throughout a 4 minute test.

2.4 VALIDITY AND RELIABILITY OF ANIMAL MODEL OF DEPRESSION

Authentication (Validity), generally explained as the procedure of dependability as well as importance of a procedure remain recognized in a particular function. Dependability is considered the ability to replicate a test intra and inter-laboratories and over a reasonable period.Authenticity standards have remained framed as well as verified for animal model of depressionand anxiety, comprising of synchronized, construct, discriminant, extrapolative and etiological face cogency (Geyer & Markou, 2001; Sarter and Bruno, 2002).

2.5 LIPOPOLYSACCHARRIDE INDUCED DEPRESSION

Majority of accessible anti-depressant remedies improve or regulate mono-aminergic neurotransmission (Li *et al.,* 2012). Recognition of new neuro-biological objectives of major depressive disorder, remains a study importance alongside a view of upcoming templates for extra effective and/or quicker performing anti-depressants (Rizvi and Kennedy, 2011; Machado-Vieira *et al.,* 2009). In line with the joining confirmation suggest that interrupted neuroplasticity performs a crucial function in major depressive disorder pathophysiology (Ota and Duman, 2012). Reduced level of neurotrophins; brain-derived neurotrophic factor, (BDNF)have been found within the brain and serum of persons with major depressive disorder condition in comparison with normal individuals (Sen *et al.,* 2008).

Moreover, the increase in hippocampal BDNF signaling facilitates the activity of regular anti-depressants (Zheng *et al.,* 2010; Schmidt and Duman, 2007). Numerous proofssuggest upsurge in the level of oxidative stress as well as nitrosative stress (ONS) as the triggersof MDD (Maes *et al.,* 2011). Research has it that, rise in the level ofROS and RNS in MDD, together with peroxidase (Maes *et al.,* 2010) as well as N.O, (Suzuki *et al*., 2001; Dhir and Kulkarni, 2011). Additionally, low concentrations of antioxidant defenses, for instance, glutathione(GSH) in the autopsy of a brain of MDD victim has been established (Gawryluk *et al*., 2011). Consequently, mechanisms ofONSare considered to be the focus of upcoming

anti-depressants (Lee *et al.*, 2006). Latest indications suggest that elevated immunoglobulin M (IgM) and immunoglobulin A (IgA) reactions hostile to lipopolysaccharide(LPS)obtained from Gram negative gut commensal in persons suffering from protracted depression (Maes *et al.,* 2012a) suggests anaction of elevated movement of leaky gutAKA Gram-negative bacteria (Maes *et al.,* 2008).

Consequently, serum concentration of inflammatory cytokines, e.g., TNFα,is increased in patients withMDD conditions (Seidel *et al.,* 1995; Sluzewska *et al*., 1996; Maes, 2008; Dowlati *et al*., 2010; Hannestad *et al*., 2011). Nevertheless, this basis remains uncertain, although a bi-directional link between pathways of negative mood and inflammation occurs. Hence, negative moods triggers an up-regulation in thelevel of systemic inflammation, contrariwise, inflammatory mediators in the peripherysignals brain towards influencing neurobehavioural changes associated with MDD (Messay *et al*., 2012).

2.6 ANXIETY

Anxiety, as an emotional response to a perceived threat, is distinguished by motor sympathetic hyperactivity, vigilance and apprehension syndromes. It is distinguished from fear which remains the normal emotional responses toward an actual otherwise sensed impending danger. Anxiety is a normal adaptive emotion since it promotes survival by provoking a person to avoid dangerous locations (American Psychiatric Association, 2013). State of anxiety is regulated through mutually facilitatory as well as inhibitory mechanisms that either favor or counter anxiety conditions. Specific cortical coupled with sub cortical brain areas are being affected by neurochemical and neuropeptide systems associated with anxiety disorders, thereby acting as targets for anxiolytics (Neumeister *et al.*, 2005). Mediators that influence norepinephrine and serotonin systems, antagonist ofneurotransmitter systems for instance, Substance P and corticotropinreleasing factor(CRF) or reduction in

glutamatergic neurotransmissions, e.g,agonists of metabotropic glutamate receptor, neurotrophic factors stimulation, likeBDNFs, are neurogenesis enhancers (Gorman, 2003).

2.6.1 CLINICAL CATEGORIES OF ANXIETY

i. Panic Disorder, a condition of tremendous worries, characterized by physical indicators such as shivering, perspiring as well as chest pain among others (Tharmalingam, 2006)

ii. Post-traumatic stress disorder (PTSD),a form of anxiety, evoked via consistent recollection of previous traumatic occurrences (Kathryn, 2003).

iii. Social anxiety disorder, characterized by recurrent fear of public attention and state fright (Lochner, 2006).

iv. Phobia, a strong dread for specific things or situations like open spaces, snakes, social interactions and flying (Iancu, 2006).

2.6.2 EPIDEMIOLOGY OF ANXIETY

Occurrence of anxiety conditions is estimated to be about 30% of mood conditions (Young *et al.,* 2004). It has been stated that shortly before and after delivery, frequencies of obsessive-compulsive disorder as well as generalized anxiety disorders(GADs) is usually prominent in females than in the overall populace (Ross and McLean, 2006). Statistically, 47.5 percent of patients with MDD experience anxiety conditions, and anxiety conditions26.1 percent also experienced major depressive disorder. However, nearly 8% of conditions seeking medication have generalized anxiety disorder (Beekman *et al.,* 2000). Panic disorder frequently occurs alongside essential hypertension as well as postural tachycardia syndrome (Roy-Byrne *et al*., 2006).

2.6.3 MAJOR CAUSES OF ANXIETY DISORDERS

Anxiety Disorders are triggered by some factors such as:

i. **Anxiety disorders and Heredity:** About 35% to 45% of traditional paradigms of conditioned fear and phobias exhibit minor heritability in humans (Hettema *et al.,* 2003).

ii. **Anxiety Disorders and Personality:** Generalized anxiety disorder (GAD) andPTSD are connected to behavior (Gamez *et al.,* 2006). Among various personalities, people with poor coping skills and low self-esteem are oftentimesvulnerable to disorders with respect to anxiety (Karatzias *et al.,* 2006).

iii. **Life Experiences:** Traumatic early life occurrences expose individuals to anxiety conditions (Gladstone *et al.,* 2006).

2.6.4 MAJOR ANXIOLYTICS; BENZODIAZEPINES (BZD)

This is a class of psychoactive drug which have its main chemical make-up as the combination of a diazepine and a benzene rings. Leo Sternbach in 1955 was foremost to the discovery of chlordiazepoxide (Librium) by chance, and its availability was noticed sometime in 1960 (Shorter*,* 2005). Benzodiazepines improve the effect of GABA at the $GABA_A$ receptor, thereby causing muscle relaxant, hypnotic, anticonvulsant, anxiolytic and sedative effects (Olsen and Betz, 2006*)*. Benzodiazepines are usually classified as either long, intermediate, or short-acting. Intermediate in addition to short-acting benzodiazepines are ideal in remedy of sleeplessness; long acting benzodiazepines are endorsed for the cure of anxiety (Dikeo *et al*., 2008*).*

2.6.4.1 MECHANISM OF ACTION OF THE BENZODIAZEPINES

Benzodiazepine actsby causing an increase inGABA function, which is a regular brain inhibitory neurotransmitter. $GABA_A$ receptors are made up of an ion channel which transmits chloride ion through neuronal cell membrane and two binding sites for GABA, whereas a subdivision of $GABA_A$ receptor complex, similarly comprise of specific attaching position for benzodiazepine (Fig 1). The mechanism through whichbenzodiazepines actposes a difficulty in its categorization as either anxiolytic alone or a sleep inducer. For instance, a sleep inducer given in low dose replicates anxiolytic effects, while benzodiazepine sold as anti-anxiety medication will, at greater dosages;stimulate sleepiness (Puri and Tyrer, 1998*)*.

2.7 ORIGIN AND DISTRIBUTION OF BREAD FRUIT

Breadfruit (A*rtocarpus altilis*) is originated from *A.*camansi Blanco and *A.marianmensis* trecul (Zerega *et al*., *2004*). The breadnut (*A.camansi Blanco*) is native to New Guinea, Moluccas (Indonesia) and the Philippines. Around 3000 years ago, breadfruit was first domesticated in the western pacific and spread throughout the tropics by migrating Polynesia where it begun to cultivate widely by pacific islanders. In the late 1700s, several seedless Polynesian breadfruit varieties and breadnut from New Guinea were introduced to the Caribbean; where breadfruit is regarded entirely as a food for the poor. Then, it was subsequently distributed throughout the Caribbean to central and South America, Africa, India, south East Asia, Madagascar, the Maldives, the Seychelles, Indonesia, srilanca, northern Australia and south Florida. In recent years, some breadnut trees have been planted in French Polynesia New Caledonia, Palau and Hawaii mainly by Philippine immigrants (Morton 1987; Ragone, 2009). In order to understand the relationship between breadfruit and wild relatives, Zerega *et. al.,* (2004) collected 254 samples of breadfruit and its relatives, among the sample 24 of them were *A. marianmensis,* 30 *A. camansi* and 200 pacific cultivars (Zerega *et. al.,*2004*).* The samples were analyzed using amplified fragment length

polymorphism (ALFP) with three different primer pair combinations. The experimental data analysis reported by Zerega *et al.*, (2004) reveal the role of *A.camansi* and *A.marianmensis* in determining the breadfruit. Then, it was later dispersed all over Caribbean, Africa and the rest of the world including, the Maldives, Indonesia, the Seychelles, Srilanca, Madagascar, south Florida and Northern Australia.

In modern times, several breadnut plants have been cultivated in France, Hawaii and Palau majorly by immigrants of Philippine origin (Morton 1987; Ragone, 2009). In order to comprehend the connection linking breadfruit and wild relatives, Zerega *et al*. (2004) gathered 254 samples of breadfruit and its relatives, among the sample 24 of them were *A. marianmensis*, 30 *A. camansi* and 200 pacific cultivars (Zerega *et. al.,*2004*).* The samples were evaluated by means of amplified fragment length polymorphism (ALFP) with three dissimilar primer pair mixtures. The investigational data analysis stated by Zerega *et al.*, (2004) exposed the role of *A.camansi* and *A.marianmensis* in determining the breadfruit.

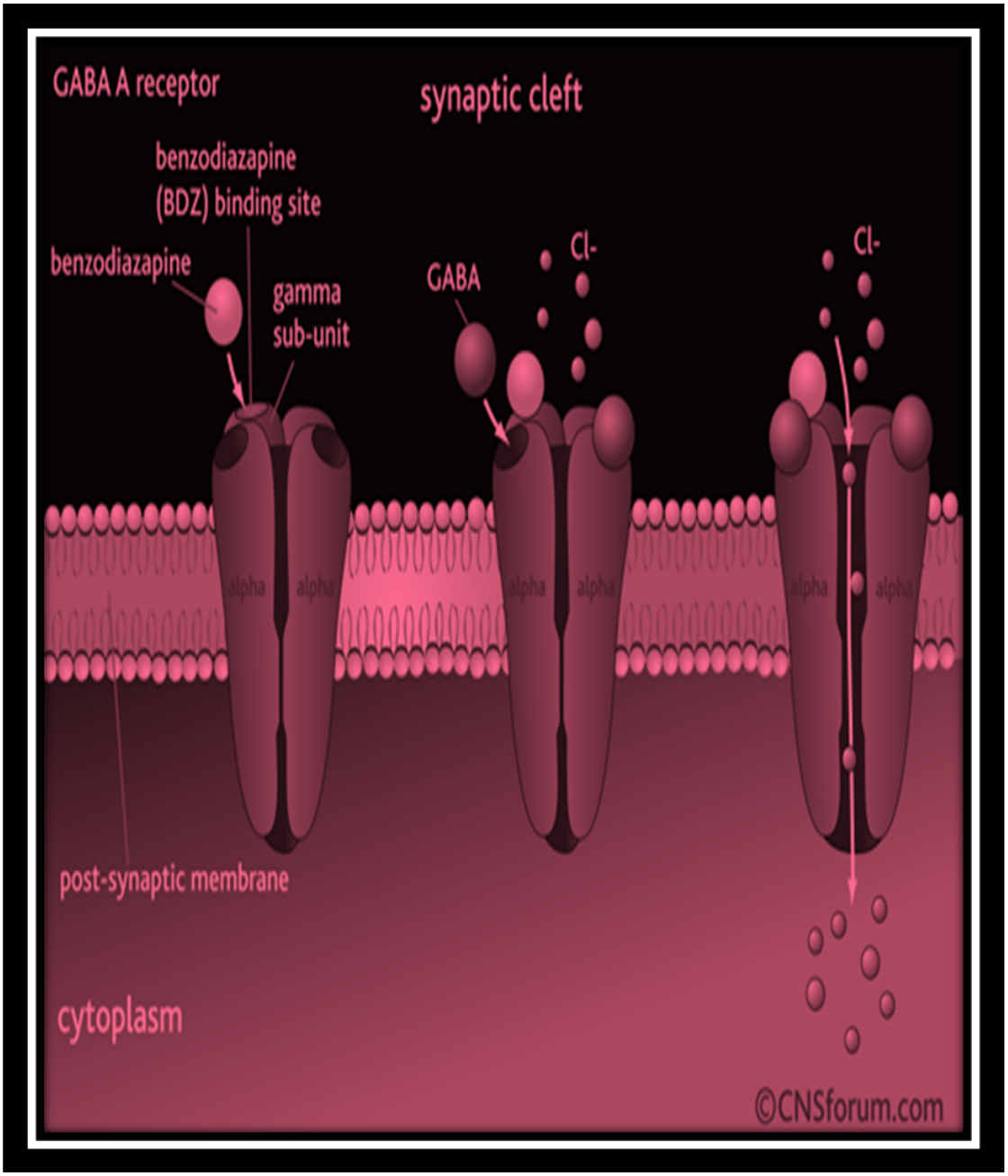

Fig 2.1: **Site of action benzodiazepines (Katzung, 2001)**

2.7.1 PHYTOCHEMICAL CONSTITUENTS OF *Artocarpus altilis*

Phytochemicals are minor byproducts of metabolism. Ample types of byproducts of metabolism are manufactured in plants. Their operation aims towards the enticement of animals otherwise to avert contamination, parasitic as well as predatory activities, although not essentially meant for important breakdown. *Artocarpus* type knows how to create a substantial amount of minor byproduct of metabolism normally abundant in phenyl propanoids namely, sterols, flavone and flavonoid. They as well yield phenolic compounds containing flavonoid, stilbenoid as well as arylbenzofuron. More than one hundred and thirty compounds are recognized within several structures of the family tree of *Artocarpus altilis*, not fewer than seventy, are developed beginning from phenyl propanoid pathway. Numerous amounts of the compounds are separated and shows that they are biologically active for instance, obstruct platelet aggregation, anti-fungal potentials, bacteriocidal, as well as prevention of leukemial units in addition to its tumor preventive action (Handa *et. al.,*2008). Nutritive components present in the plant seed possesses fat, carbohydrate, water, protein, niacin, iron, thiamine, phosphorus coupled with calcium (Rahul, 2013).

Breadfruit *(Artocarpus altilis)* possesses certain organic components namely:, moracin, cynomacurin, morin, dihydromorin, oxydihydroartocarpesin, Artocarpetin, Noraartocarpetin, artoflavon, cycloartinone, oratocarpanone, oxyresveratrol, artoindonesianin and many others (Solanki and Nagori, 2012). The specie similarly comprises of kazinol A, (Mukesh, 2014).

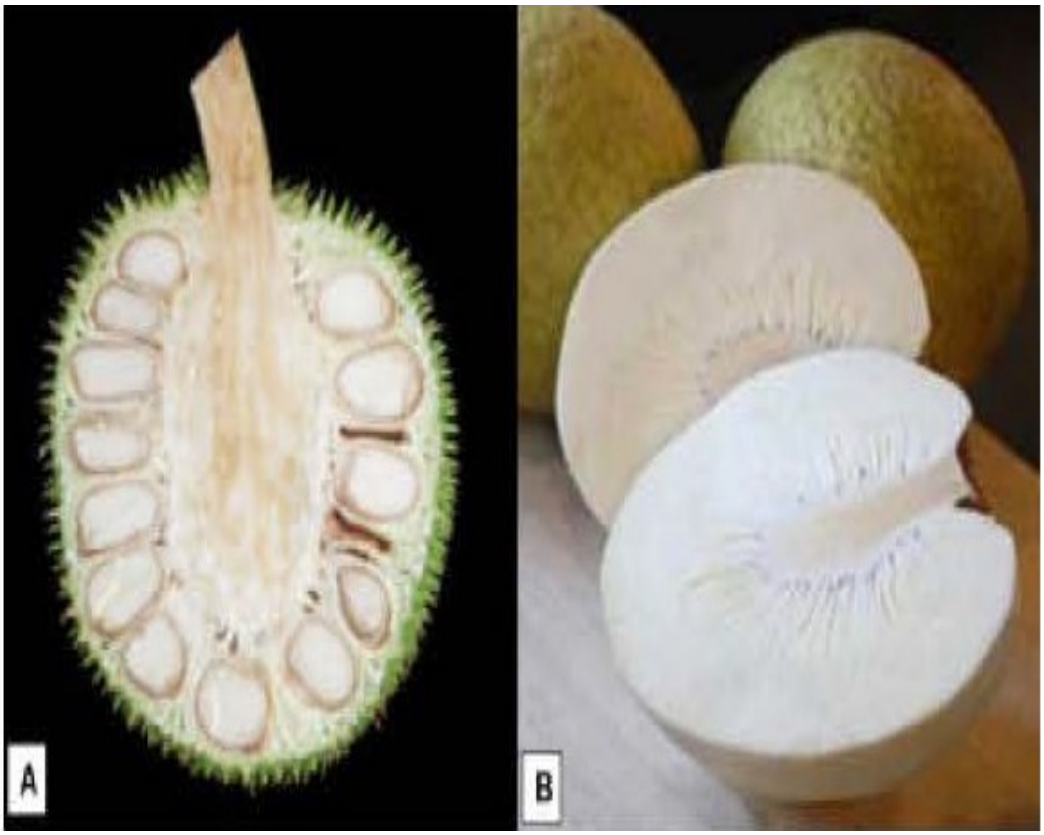

Fig 2.2: A and B showing typical *Artocarpus altilis* with seeds (Source; Breadfruit Institute, National Tropical Botanical Garden, Hawaii).

Fig. 2.3: *Artocarpus altilis* in various stages of preparation (Source; Researcher, 2016).

2.7.2 THERAPEUTIC EFFECT OF BREADFRUIT

The Heterogeneous significance of various portions of breadfruit (inner bark, leaf tips, and latex) comprises of cosmetics, nutriment, clothing material, medication, building provisions and animal food (Pradhan *et al*., 2013; Ragone 1997; Donsing *et al*. 2008). Reduced latex concentration is ingested on the inside, to remedy Gastric ailments such as diarrhoea. Juice obtained from squashed stalks along with leave is utilized as cure to ear illnesses. Roots of breadfruits are caustic and therefore, consumed as a cleansing agent. Yellowing of the leaf in West Indies is applied to tea to decrease high blood pressure and get rid of asthma (Ragone, 1997).

Heated leaf ash can be applied to alleviate splenomegaly condition (Morton, 1987). Varieties of *Artocarpus altilis* remained scrutinized on its anti-microbial actions through numerous investigators (Ragasa *et al.,* 2004; Shanmugapriya *et al*., 2011). Roots and stem barks extracts exhibited anti-microbial actionagainst Gram positive bacteria and possess potentials capable of curing of tumors (Seaforth *et al*., 1983; Sundarrol *et. al.,*1993; Ragone, 1997). Chromatographic investigation of bread-fruit showed excessive constituents of carbohydrates, fatty acids, and amino acid (Golden *et. al.,*2001).

2.7.3 IMPORTANCE OF BREADFRUIT

Breadfruit has long been an essential primary crop of Polynesia and has been cultivated extensively in tropical pacific, Caribbean and African countries (Ragone, 1995*).* The fruits are tremendous source of carbohydrates, vitamins (Parrotta, 1994) and low fat. Fruits can be consumed as baked, roasted, boiled, fried and even steamed (Ragone*,* 2003). Despite its carbohydrate richness, breadfruit is replaced partly with wheat dust within several snack, bread, as well as pastry stuffs. Additionally, it is a good source of fibre, calcium, copper, iron,

magnesium, potassium, thiamine and niacin. Certain forms serve as a reliable source of antioxidants as well as carotenoids.

Bread-fruit trees possess multiple utility value, literatures propose that pacific islanders use all parts of the breadfruit particularly the leaf tips, inner bark and latex as traditional drug. The timber remains light in weight, elastic and also repels termite's bout. It is used for buildings, constructing small canoes, making handicrafts, drums and also as firewood (Ragone, 1997; Morton, 1987; Whisler, 1988). The bark is used to cureheadaches while roots are strong astringent and used as a purgative. In Caribbean, the senescence leaves were utilized to maketea and also, to lowerblood pressure; it is also believedto regulatediabetes. Additionally, in Taiwan, the leaves of breadfruit are used to cure ulcer disorderand fevers. However, in some Polynesian island, the leaves are used as platters to dish upfood and as cattle feed (Whisler, 1988). Latex is frequentlyused to curebroken bones, sprains, sciatica etc. this versatiletree also provides materials for fabric, glue, animal feed, insect repellent compounds and lotsmore.

2.7.4 BIOLOGICAL ACTIVITIES OF *ARTOCARPUS ALTILIS*

i. Anti-austeric Agent

The preferential cytotoxic potentials of MEAA against cancer cells of human pancrease (PANC-1) in poor nutritive states at 50µg/mL have been documented (Nguyen *et al.,*2014). About 8 new granulated dihydrochalcone called sakenin A-H (1-8) were isolated alongside 4identified compounds (9-12) on MEAA, withsakenin F (6) also H (8) are recognized effective cytotoxics.

ii. Antiatherogenic Property

According to the findings of Oluwatosin and Olubukola (2014), MEAA using questran as standard,exhibited significant anti-atherogenic potentials as well as enhances systems of

antioxidantsinrats with hypercholesterolemia,through thecapability of initiating a considerablerisein HDL-C and enrichedantioxidant system as well as produce favourable lipid parameters.

iii. Skin Lightening Agent

Crude methanol extract, active fractions and isolated compounds of *A. altilis* were found to possess good dendrite elongation actions when investigated for dendrite elongation potentials using melanocyte cells (B16F10). These active compounds have been suggested to possess skin lightening ability and can be used in skin care (Gottumukkala *et al.,*2013).

iv. Antioxidant Agent

Horng-Huey and co-workers in2013 estimated the anti-oxidant actionsof flavonoid isolatesfrom cortex and heartwood of *A.altilis* by assessingthe inhibitory properties on melanin biosynthesis and mushroom tyrosinase invitro.Potentials of prenylated flavonoids comprising isoetin, hydroxyprenyl, 10-oxoartogomezianone, furanocyclocommunin as well as Isocyclo-artobiloxanthone wereassessed collectivelyalongside twelve additionally identifiedmixtures in curbing DPPH, ABTS+ essential cation, in addition to superoxide anion (O2), coupled with thecapabilitiesof inhibitingmelanin as well as tyrosinase production. They concludedthat these flavonoids are appropriate as anti-oxidants and can also function as skin whitening agentsthough morestudies as to unravel their possible mechanisms of action are encouraged.

v. Alpha (α) Glucosidase and Alpha (α) Amylase Inhibitor

According to Sindhu and coworkers in (2013), methanol extract of some species of *Artocarpus,* alongside *CinnamomumPiper betel* and *zeylanicum* were able to inhibit alpha(α) glucosidase and its enzyme effectively invitro,though, just*A. heterophyllus* is advantageous withinpost-prandial hyper-glycaemia management.

vi. Antimicrobial Agent

In line with the findings of Chinmay and coworkers in 2013, diverse solvent extracts comprising ethyl acetate, petroleum ether and methanol of *A. altilis* leaves exhibits antimicrobial activities, methanol extract in higher concentration having the highest antimicrobial potential. On the other hand, ethyl acetate and petroleum ether leaves extract exhibited enhanced antimicrobial potentialsin lower amounts.

vii. Antihypertensive

Nwokocha and coworkers in 2012 verifiedthat aqueous extract of *Artocarpus altilis*displays hypotensive as well as negative chronotropic properties in antagonism of calcium ion (Ca^{2+}) channel and also inAlpha (α)-adrenoceptors.

Rats were introducedto propranolol, mepyramine, atropine and methyl ester N (G)-nitro-L-arginine. The outcomeindicated modestpreventions of cytochrome P450 (CYP2D6 and CYP3A4) enzymatic actions.

viii. Mosquito Deterrent

Jones and coworkers in2012 exploredchemical constituents in powdered male inflorescence of bread-fruit, liable to mosquito(*Aedes aegypti)* prevention. This research substantiatedthe male bread-fruit flower as well as fatty acid has capability to repel mosquito.

2.7.5 PHARMACOLOGICAL USES OF BREADFRUIT

i. Anticoagulants and antiplatelet Activities

Basavraj(2013) suggested that bread-fruit might improve the properties of additional herbs in the increase in risks involving blood loss by preventing the formation of thromboxane, an inducer of platelet aggregation.

ii. Antineoplastic effect

Correspondingto workshop assessment, chemical constituentspresent in the leave of *A. altilis* demonstrates cell toxicity as well as programmed cell death potentials on human beings' cancerous cells by elevating manifestationof programmed cell death inducing proteins like FasL, Fas, and p53 proteins. Leave extract of bread-fruit might purportedly possess combined potential on antineoplastic herbs or supplements (Somashekhar, 2013)

iii. Inotropic effect

According to the discovery of Naira (2013), leaf extract of *A. altilis* exhibits a mild inhibition on the force of contraction to decrease left ventricular pulse pressure as well as decreases the excitability of the right ventricular myocardial bands.

CHAPTER THREE

MATERIALS AND METHODS

3.1 APPARATUS

Apparatusutilized in the present investigation includes;

Zhengya weighing balance, Plastic cages, Latex glove, Rubber gloves, Masking tape, Rubber transporting basket, Syringes (1mL, 2mL, 5 mL, 10mL, and 20mL), EPM stand, EZM stand, LDT Box, OFT Box, TST Stand, FSTPlexiglas and stop watch.

3.2 DRUGS AND CHEMICALS

Chemicals utilized in thecourse of this investigation remained of analarrating and gotten Via Sigma Company, Chemical division. St. Louis, USA.Drugexpended during the study were newly made earlier as well as administered at a dose 10 ml/kg. Calculation of dosages achieved based on the animal's body weight and expressed as mg/kg p.b.w.Drugs used included: metergoline-Non-selective $5HT_2$ receptor antagonist (4 mg/kg, *intraperitonearly*), sulpiride, prazosin-antagonist of (α_1-) adrenergic receptor (62.5 µg/kg, *i.p.*), diazepam (DZP) (1 mg/kg, (*per oral)*, imipramine (IMP) (10 mg/kg, *i.p.*) as well as lipopolysaccharides (LPS) of E. coli, strain 055:B5.

3.3 EXPERIMENTAL ANIMALS

A total of 252 Adult male Swiss mice weighing between 20-25g, bought fromthe Central Animal House, College of Medicine, University ofIbadan, Ibadan. Mice were housed under normal environmental conditions and permitted access to drinking water as well as mice cubes from Ladokun Feeds Nigeria Limited, Ibadan, Nigeria, ad libitum. Animals were left to acclimatize within fourteen days prior to commencement of the experiments.

3.4 PLANT MATERIALS

Fresh fruit seeds (10kg) of *Artocarpus altilis* (breadfruit) were collected, identified and authenticated at the FRIN, in Ibadan, Nigeria, where FHI No: 110483 was given as the voucher specimen number. The fresh fruit seed of *Artocarpus altilis* (breadfruit) wereair dried at 37^{o}C and thenground.

3.5 METHODOLOGY

3.5.1 PREPARATION OF PLANT EXTRACT

Powdered dried *Artocarpus altilis*was extracted by cold extraction for 72 hours using methanol (MeOh). The methanol extract provided a semi-solid residue (Methanol extract; 7.2kg) and the percentage yield was 96%.The powdered *Artocarpusaltilis* was soaked in a glass container and left for a period of 72 hours after which the powdered dried breadfruit settled and the clear liquid was decanted and then evaporated using rotatory evaporator. The extract was evaporated to semi-solid form (Odec, 2001).

3.6 ANXIETY AND DEPRESSION-LIKE BEHAVIOURAL STUDIES

The drugs (reference drugs; imipramine hydrochloride and diazepam (10 mg/kg; *p.o*and (1 mg/kg. (*i.p)*.)respectively weregiven to the mice1 hour prior to the experiment. The drugs and extract were dissolved in normal saline. Graded doses of MEAA(50, 100 and 200 mg/kg, *p.o*.), in addition to IMP (10 mg/kg, *p.o*.) were given to mice at least 1 hourprior to TST as well asFST. The highly efficacious dose 200mg/kg, was usedfor elucidation of the mechanism(s) of action(s). Animals were subjected into behavioural studies of anxiety and depression as explained below.

3.6.1 DEPRESSION MODELS

The potentials of bread fruit on depression was investigated using two investigational methods, which includes FSTas well as TST. Test methods comprised of five (5) groups of six (6) miceeach. The first of the groups, referred to as control,was given vehicle only (Normal Saline). Mice in groups 2-4 were given MEAA(50, 100 and 200 mg/kg, p.o) whereas Group 5 was given imipramine (10 mg/kg, i.p) as the standard antidepressant drug. Investigation was executed one hour after treatment.

i. Forced Swim Test (FST)

The FST (Fig. 3.1)was executed by adherence to method of Porsolt *et al.*, (1978). FST apparatus was made up of transparent Plexiglas container (20 cm X 12 cm) filled up with water to the 15 cm depth at a temperature of 25±1^{o}C. Investigational animalswere exposed individually to swimming on the plexiglas for 15 minutes 24 hours to the test, and arbitrarily allocated to five (5) groups of six (6) mice (n=6). One hour following treatment, animals were forced to swim for a period of 6 minutes and immobility duration wasdocumented for the duration of the last five minutes with the use of a stopwatch. Animal is thought to be immobile as it makes just movement required in keeping its head afloat.

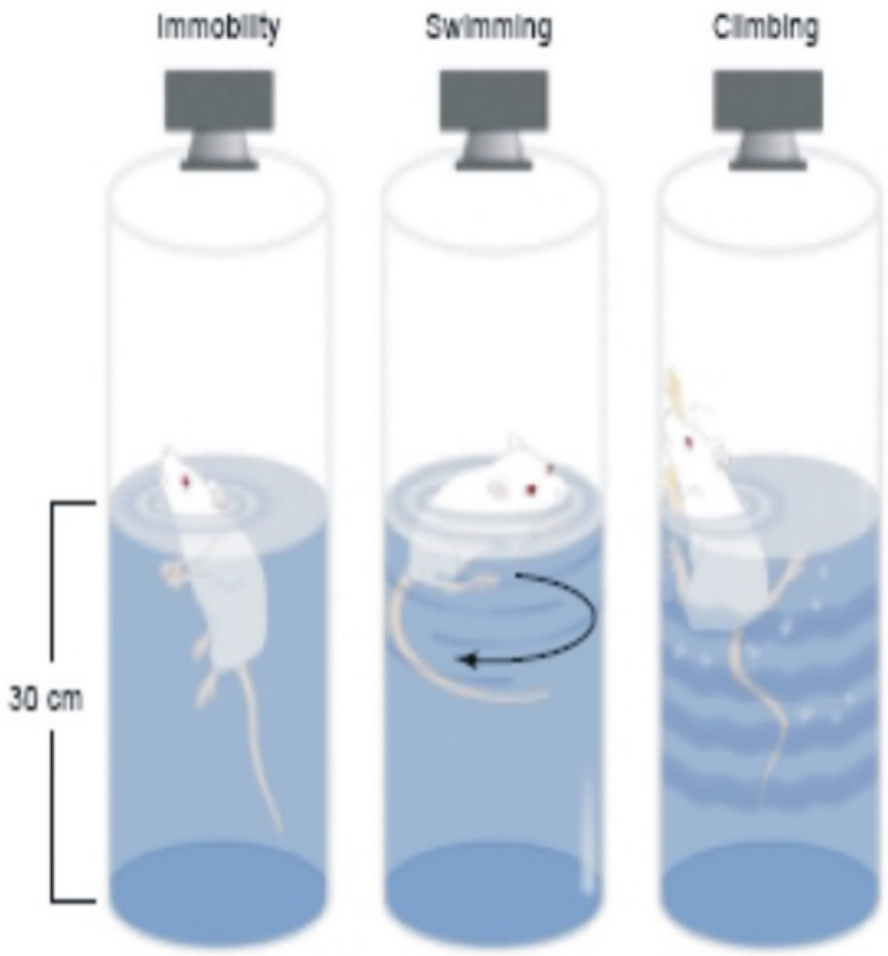

Fig. 3.1: Shows the forced swimming test Plexiglas and various parameters evaluated(www.google.com, assessed 10thMay, 2015)

ii. Tail Suspension Test (TST)

Steru *et al.*, (1985) were the first to demonstrate the TST (Fig. 3.2). Test animals (mice)remained separately and were independently hanged up in the air 60cm overhead the surface of an elevation on a metallic bar by a paste tape positioned 1cm outside the apex of the mice tail. Period of stillness known as immobility interval noted within final five (5) minutes of a six (6)-minute experiment. Mouse was judged to be immobile simply once it hangs inactively and are totally stationary or immovable (Cryan *et al.*, 2005).

Fig. 3.2: Image description of the tail suspension test, the mice suspended and ready for action (www.google.com, assessed 10th May, 2015)

3.6.2 ANXIETY BEHAVIOURAL STUDY

Anxiety was investigated in three investigational techniques. The assessment process consists of 6 mice in each of the 5 groups. Group 1 served as the control, groups 2-4 took MEAA (50, 100 and 200 mg/kg) while group 5,which served as the positive control received diazepam (standard drug, 1mg/kg). Experiment was performed 30 minutes after treatment.

Elevated Plus Maze (EPM)

The EPM (Fig. 3.3) test was originally designed by Montgomery in 1958 (Montgomery, 1958). It was designed to evaluate anxiety-like behaviour in laboratory animals. The device is made up of 2 open as well as 2 close parts radiating from a pivotal stand, forming a plus sign raised up to an altitude of 40 cm overhead the base. Experimental mice remained midpoint stage thirty minutes following corresponding medication, each, facing the opened arms. The number of time the animal enters the opened and closed arms of the EPM and the period it expended within the opened and close arms were recorded within 5 minutes. Device was thoroughly wiped by means of a moist and dry towel for each trial to eradicate any form of residue or stench (Kulkarni and Reedy,2009).

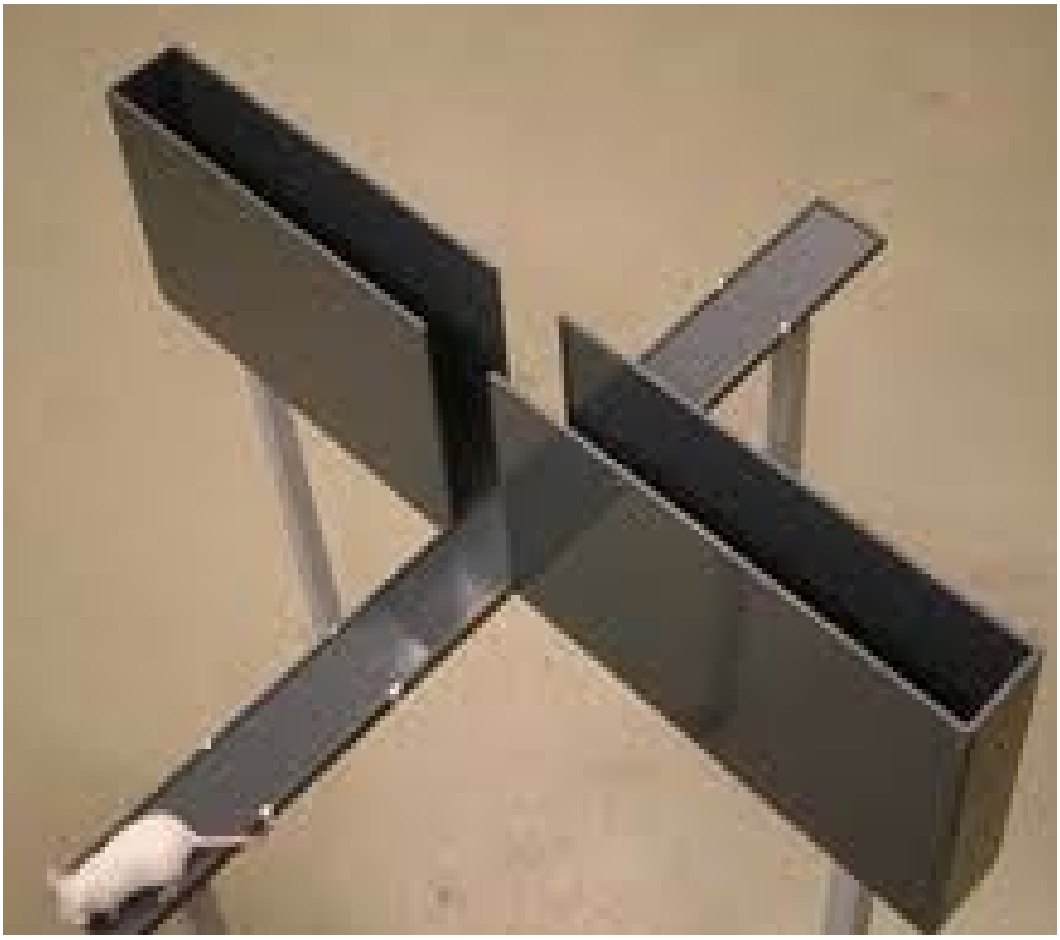

Fig 3.3: The Elevated plus Maze stand (www.google.com, assessed 10th May, 2015)

i. Elevated Zero-Maze (EZM)

The EZM(Fig. 3.4) is a modification of the plus maze. It was constructed of black acrylic in a circular track 10 cm wide, 105 cm in diameter, and elevated 72 cm from the floor (San Diego Instruments, San Diego, CA). The maze was divided into four quadrants of equal length with two opposing open quadrants with 1 cm high clear acrylic curbs to prevent falls and two opposing closed quadrants with black acrylic walls 28 cm in height. A 5 min trial under the same lighting conditions as in the Plus maze began with the animal placed in the center of a closed quadrant. Dependent measures were the same as for the Plus maze except that there was no center region. Between trials, the maze was cleaned with 70% ethanol.

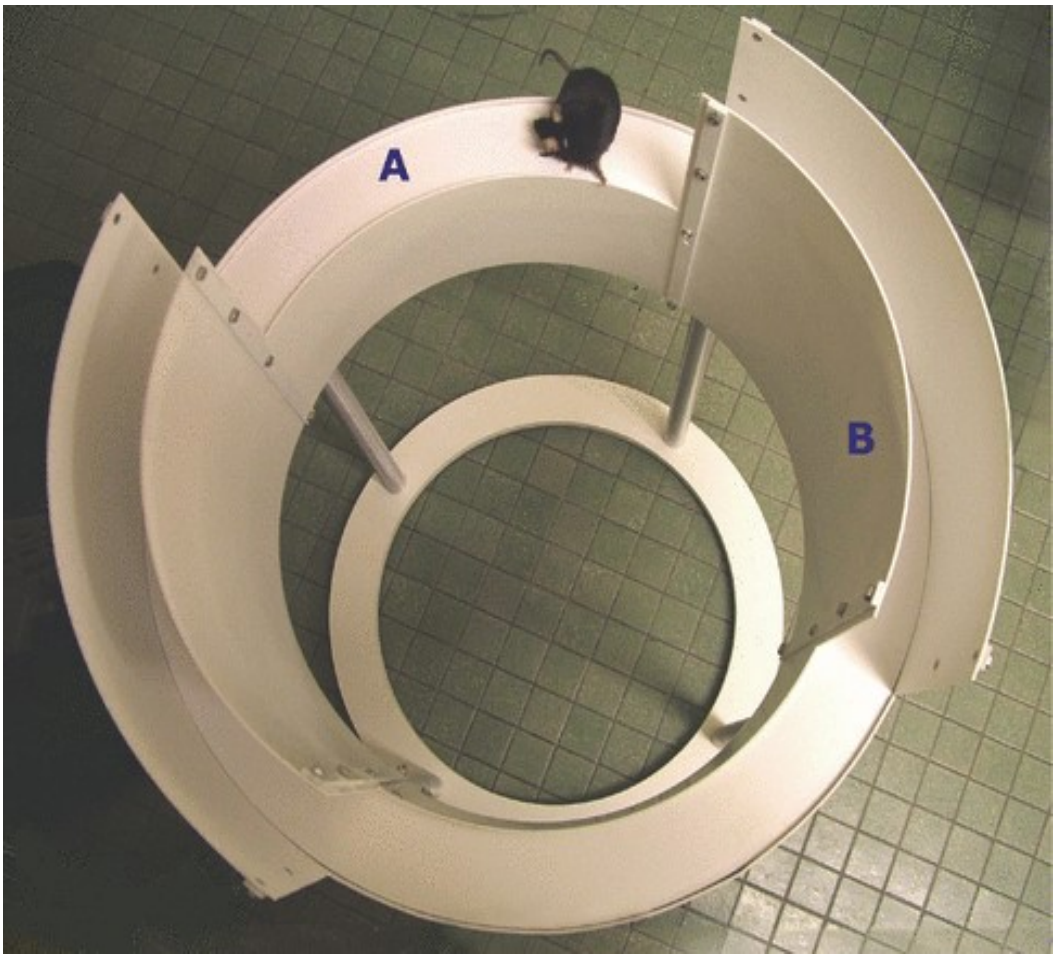

Fig 3.4: The Elevated zero Maze stand (www.google.com, assessed 10th May, 2015)

ii. Light-Dark Test (LDT)

L&D equipment (Fig. 3.5) consists of Plexiglaslike a box made up of partitions 1 and 2 (20cm × 20 cm). Partition 1 is bright whereas the other remained dim. On each testing, animals were individually positioned by the intersection of the L&D, opposite the bright partition and the time it spends around the bright partition, as well as total sum of entry into eachpartitionwas recorded for 5minutes (Young, 1991). The box was dry-cleaned with wet tissue paper dipped in 10% ethanol solution in theend of each test. One off shot MEAA (50, 100and200 mg/kg) and DZP (1mg/kg, i.p), given one hour prior to test for MEAA and 30 minutes for diazepam.

Fig 3.3: Image showing the Light and dark test box(www.google.com, assessed 10th May, 2015)

iii. Open Field Test (OFT)

Locomotor (ambulatory) behavioural activitieswere measured inside an open field(Fig. 3.6)It is made up of a wooden box with size of 40×60×50 cm and is used for the evaluation of a lot of paradigms including anxiety, depression, exploration and locomotion due to its large central arena. Its field has walls to prevent the animals from escape tendencies. The field is distinct by a grid and square crossings. Number of line crossing and time spent moving are applied to evaluate the activities of the rodent.The open field test is a widely used model of locomotor activities developed to evaluate emotionality in animals and is based on subjecting an animal to an unknown environment whose escape is prevented by surrounding walls. The animal is placed into the center the apparatus number of line crossings is recorded over a period of 6 minutes duration.

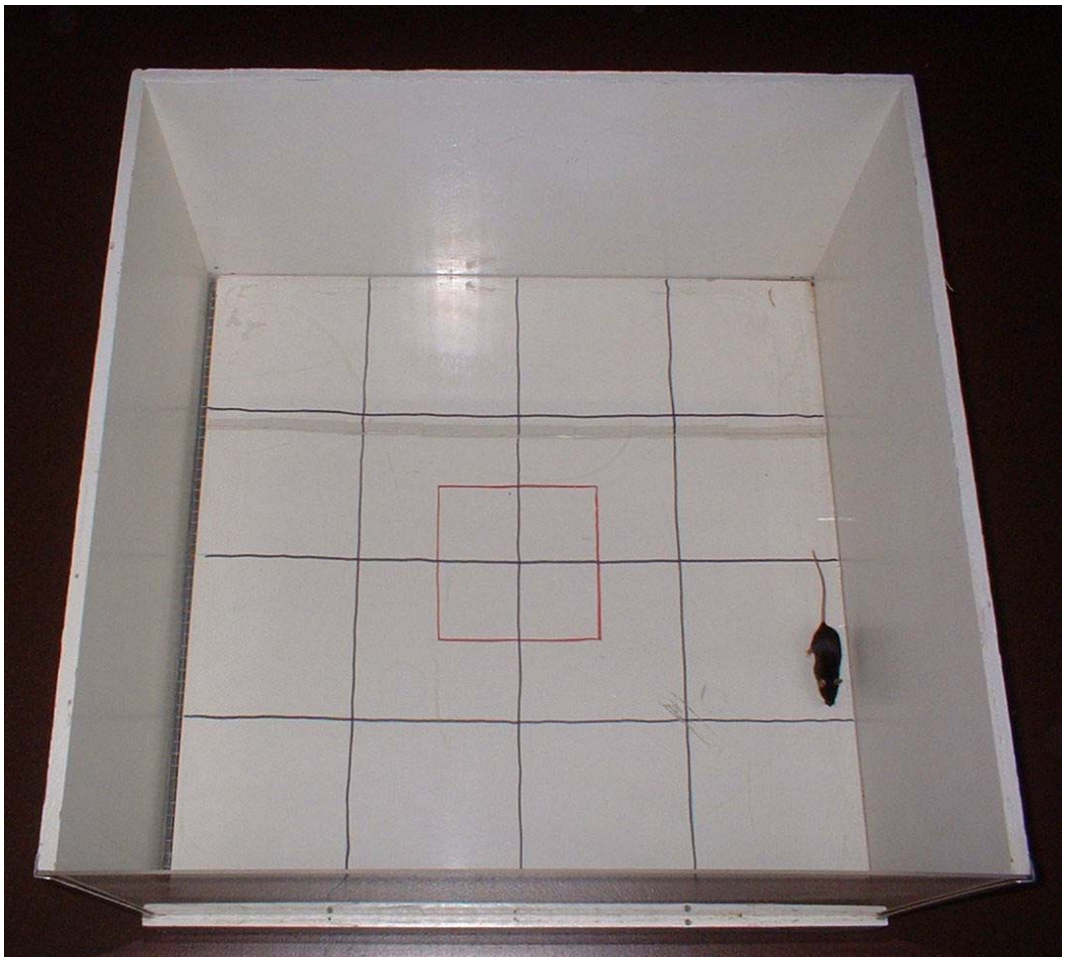

Fig. 3.6: Image showing the Open field Test board with mice exploring the various lines

(www.google.com, assessed 10th May, 2015)

3.7 Determination of Mechanism of Action of Methanol Extract of *Artocarpus altilis* using Tail Suspension Test

The most effective dose of methanol extract of *Artocarpus altilis* was used for the elucidation of the mechanism through which the methanol extract of *Artocarpus altilis*exhibits its anti-depression-like actionusing the following blockers: Prazosin (1mg/kg i.p alpha-1-adrenergic receptor antagonist), sulpiride (50mg/kg i.p.,dopamine D_2 receptor blocker) and metagoline (4mg/kg i.p., $5HT_2$ receptor antagonist). Thirty mice were used in this phase. They were grouped into five (5) groups of six (6) animals each and were administered;

- Group 1: Control (Normal Saline) (10 mL/kg)
- Group2: ED MEAA (200 mg/kg)
- Group3: Prazosin (1 mg/kg)+ ED MEAA (200 mg/kg)
- Group4: Sulpiride (50 mg/kg) + ED MEAA (200 mg/kg)
- Group5: Metergoline (4 mg/kg) + ED MEAA (200 mg/kg)

This was followed by TST one hour after the administration of the various treatments as described in section 3.6.1.

3.8 LIPOPOLYSACHARIDE (LPS)-INDUCED DEPRESSION-LIKE BEHAVIOUR

LPS-induced depression test was carried outusing 24 mice.Graded doses of MEAA (50, 100 and 200 mg/kg) and imipramine (10 mg/kg) were given to four groups of animals30 minutes before the administration of LPS (0.5 mg/kg, i.p) across the groups. Twenty four hours later, the mice were screened for depression-like effects using the forced swimming test and open field test as described. The animals were sacrificed immediately after the behavioural studies and their brains were used for biochemical assays.

3.7BIOCHEMICAL DETERMINATIONS

After the behavioural studies in the LPS-induced depression model, the animals were sacrificed, their brain harvested, homogenized and centrifuged for determination of protein, nitrite, reduced glutathione (GSH), thiobarbituric acid reactive species (TBARS) and superoxide dismutase (SOD).

i. Determination of Protein contents using the salt/alkaline extraction

The salt/alkaline extraction was performed as described by Mæhre *et al*., (2016), with minor modifications. Briefly, 0.5 g ofbrain tissuewas homogenized with 30 mL of 0.1 M sodium hydroxide (NaOH) in 3.5% sodium chloride (NaCl) using an UltraTurrax homogenizer (IKA Werke GmbH, Staufen, Germany). The homogenates were incubated at 60 °C for 90 min before centrifugation at 4000× *g* for 30 min at 4 °C. The supernatants were frozen and kept at −20 °C and analysed using the amino acid analysis method.

ii. Nitrite Assay

Nitrite was assayed according to the method earlier described (Green *et al*., 1982). It centred on the production of nitric Oxide. Concisely, 100 millilitres of supernatant nurtured

(incubated) in 100 millilitres of the Griess reagents, comprised of uniform amount in the ratio of 1:1:1:1 in a one (1) percent sulfanilamide liquefied in one (1) percent H3PO4, 0.1% N-(1-naphthyl) thylenediamine dihydrochloride as well as purified water at a temperature of 37^{o}c over a period of ten (10) minutes. Measurement of absorbance was done at 560 nanometre within a microplate reader.Compositionof Nitrite verified byuniversal nitrite curve produced via the use of NaNO2 (varying from 0.75 to 100 mM) as standard and was stated as mM/mg tissue.

iii. Reduced Glutathione (GSH) Level Determination

Elman's method was utilized to estimate the brain levels of GSH (Elman, 1959). Tissuesof the brainweakened within EDTA of 0.02 M buffer solution (Ten (10) percent weight by volume) and combined to a fifty (50) percent of trichloroacetic acid (TCA) solution. At the end of the centrifugation (3000 rpm/15 min) process, homogenate supernatants gathered and combined with a 0.4 M triseHCl buffer solution at a pH of 8.9 and 0.01 M containing 5, 5-dithiobis (2-nitrobenzoic acid (DTNB)). Resultant yellow colouration was taken at 412nm with the help of spectrophotometer. Outcomes, computed on the grounds of glutathione standard curve,measured in ng of GSH/g wet tissue.

iv. Thiobarbituric Acid Reactive Species (TBARS) Level

Lipid peroxide formation was evaluated via measurement of TBARSin the homogenate (Draper *et al.,* 1993) as a pointer to production of ROS. Samples were combined with 1 mL of 10 percent TCA as well as 1 mL, 0.67 percent TBA, and heat afterwards in a bath containing boiling water for fifteen (15) minutes and is instantly preserved using cold reservoir containing ice. Peroxidation of Lipid was measured by the absorbance at 532 nm and measured in mmol of malonaldehyde (MDA)/g tissue.

v. Superoxide Dismutase (SOD)Activity Determination

The SOD was estimated by the use of a RANSODkit (Randox Labs, Crumlin, UK). Both Xanthine oxidase and Xanthine were employed in the generation of superoxide radical that usually reactsalongside 2-4-iodophenyl-3-4-nitrophenol-5 phenyl tetrazolium chloride(INT) so as to form red formazan dye. Substrate concentrations were 0.075 µmol/L for xanthine and 0.037µmol/L for INT. SODhinders the reaction through the conversion of superoxide radicals to O_2. An SODcomponent prevents the frequency by whichINT is decreased by 50 percent within a complicated arrangement together with xanthine/xanthine oxidase. As a result of theminute linearityrange of the test, sample concentration reducedso as forpercent inhibition to decrease within the range of 30 to 60 percent. Standard curve was then prepared with a cue from the already specified standard available within the kit, then value of supernatantdepicted fromthe curve. Activities of SODwithin supernatant measuredat 505 nm on a Shimadzu UV-1201V spectrophotometer. Whileoutcomes measured into SOD ng/mL protein (Delmas-Beauvieux and Clerc, 1995).

3.9 STATISTICS AND ANALYSES

GraphPadprism (GPP) 7 Software was used to analyzed data and results stated as mean ± SEM. Comparisons done by the use of one-way ANOVA followed byNewman Keuls'post hoc multiple comparison.

CHAPTER FOUR

4.0 Results

Effect of Methanol Extract of *Artocarpus altilis*on Number of Open Arms Entries in Elevated plus Maze

The Potentials of methanol extract of *Artocarpus altilis*(200 mg/kg) and Diazepam (1 mg/kg)showed significant ($p<0.05$) increase in number of open arms entries in elevated plusmazewhen compared with the control as shown in Figure 4.1.

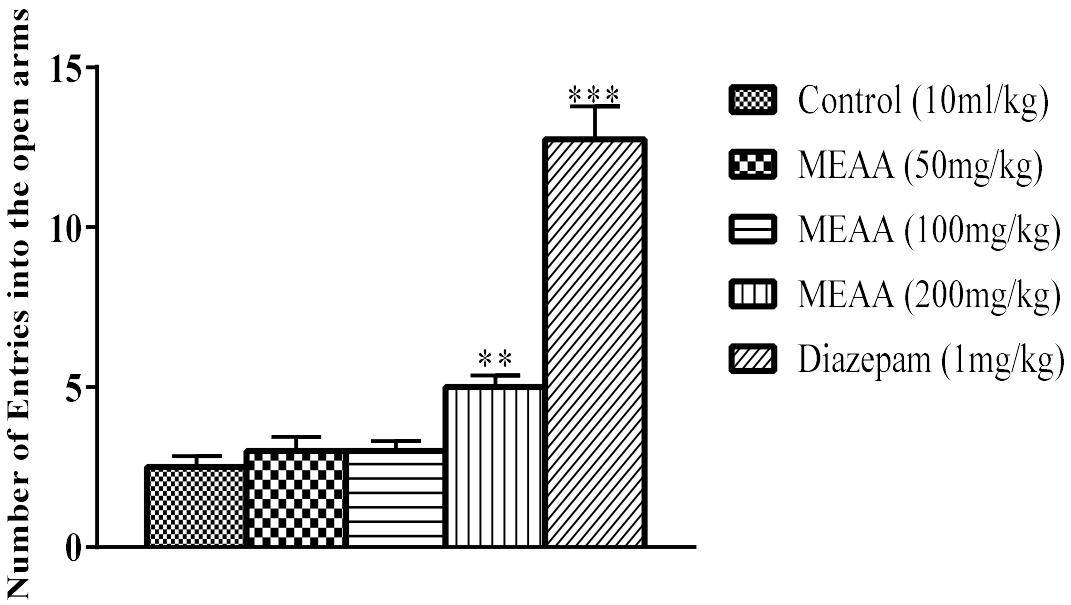

Fig. 4.1: Behavioural Potentials of Methanol Extract of *Arthocarpus altilis*as well as Diazepam on Anxiety Status of mice in Elevated plus maze. Resultspresented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls'post hoc test. **p<0.01, ***p<0.001 comparedwith the control.

Effect of Methanol Extract of *Artocarpus altilis* on Time Spent in the Open Arms in Elevated plus Maze

The potentials of methanol extract of *Artocarpus altilis*(200 mg/kg)and Diazepam (1 mg/kg)were evaluated using the elevated plus maze. Figure 4.2showed significant ($p<0.05$) increase in time spent in the open armswhen compared with the control.

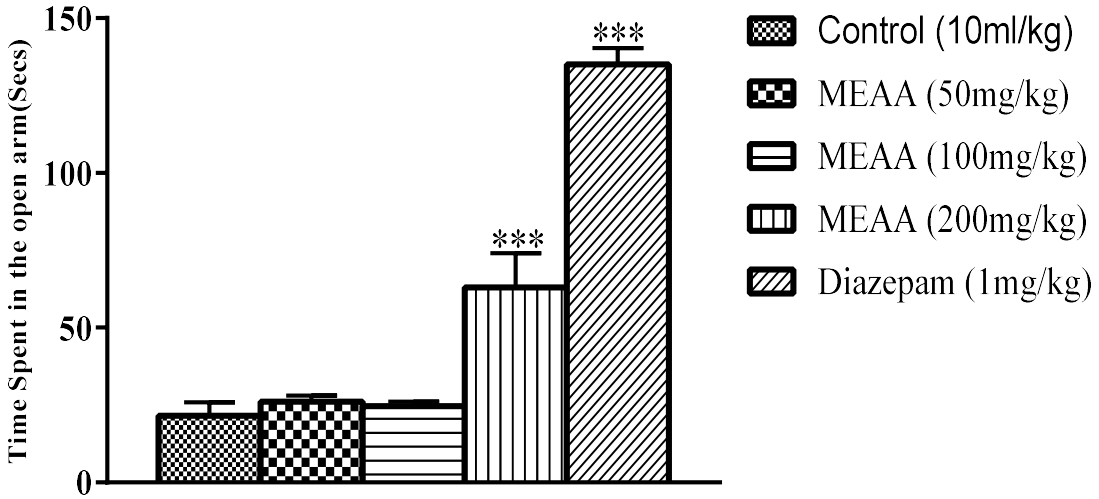

Fig 4.2: Behavioural Potentials of MEAA as well as Diazepam on Anxiety Status of mice in EPM. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test. ***$p<0.001$ compared with control.

Effect of Methanol Extract of *Artocarpus altilis*on Number of Open Arms Entries in Elevated Zero Maze

The potentials of methanol extract of *Artocarpus altilis*(200 mg/kg)and Diazepam (1 mg/kg)were evaluated using the elevated plus maze. Figure 4.2 showed significant ($p<0.05$) increase in thenumber of open arms entries when compared with the control.

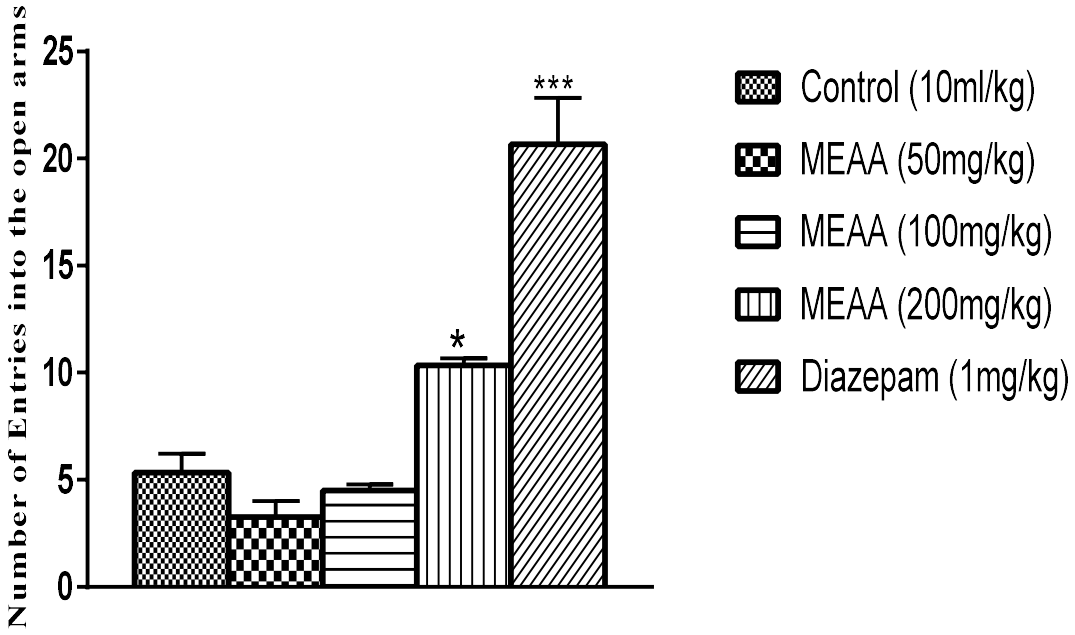

Fig 4.3: Behavioural Potentials of MEAA as well as Diazepam on Anxiety Status of mice in EZM. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test. *p<0.05, ***p<0.001 compare with the control.

Effect of Methanol Extract of *Artocarpus altilis* on Time Spent in Open Arms in Elevated Zero Maze

Methanol extract of *Artocarpus altilis*(50-200 mg/kg)and diazepam (1 mg/kg)had nosignificant ($p > 0.05$) difference in the amount of time spent in the open arms of the elevated zero maze when compared with control (Fig. 4.4).

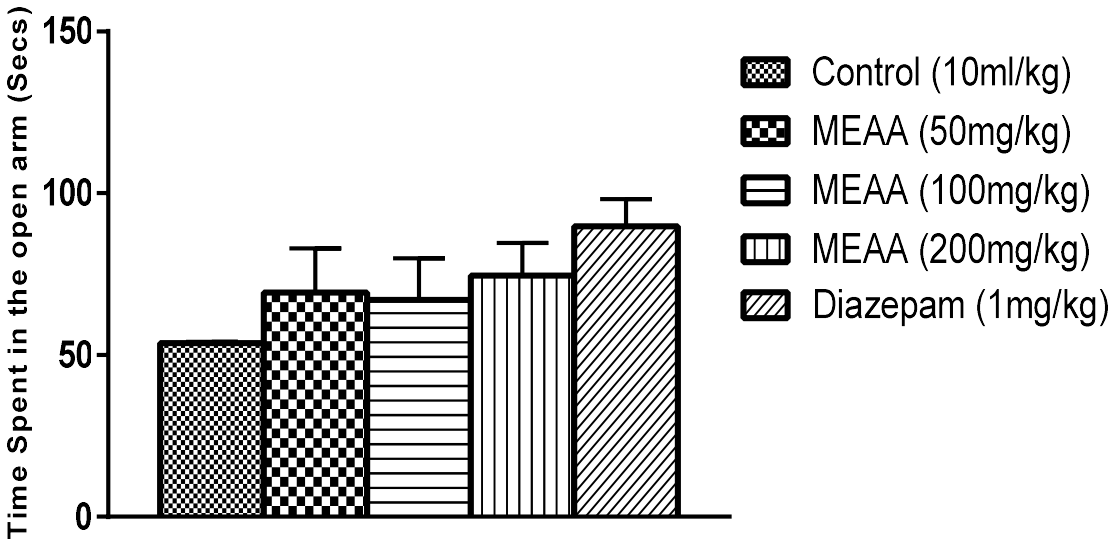

Fig 4.4 Behavioural Potentials of MEAA as well as Diazepam on Anxiety Status of mice in EZM. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test.

Effect of Methanol Extract of *Artocarpus altilis* on Number ofEntries intothe Light Chamber in Light Dark Test

The potentials ofmethanol extract of *Artocarpus altilis*(200 mg/kg)and Diazepam (1 mg/kg)were evaluated using light dark test. There was a significant ($p<0.05$) difference in number ofentries into the light chamber inwhen compared with the control as shown in Figure 4.5.

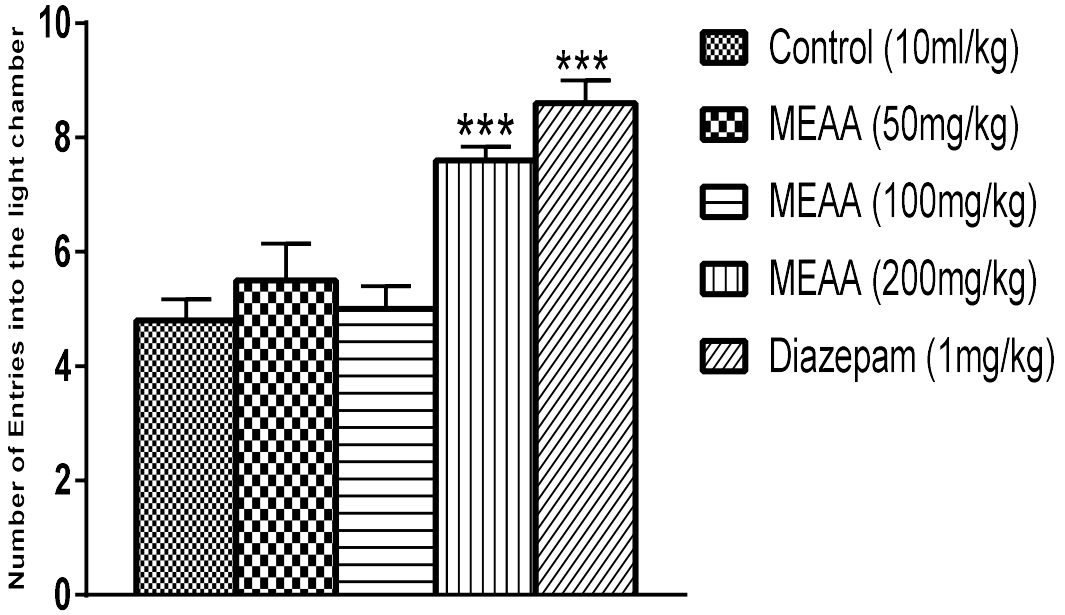

Fig 4.5: Behavioural Potentials of MEAA as well as Diazepam on Anxiety Status of mice in LDT. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test. ***p<0.001 compareto control.

Effect of Methanol Extract of *Artocarpus altilis* on Time Spentin Light Chamber in Light Dark Test

As shown in Figure 4.6, methanol extract of *Artocarpus altilis*(100 and 200 mg/kg)and diazepam (1 mg/kg) significantly ($p<0.05$) increased the amount oftime spent in the light chamber of the lightdark box apparatus when compared with control.

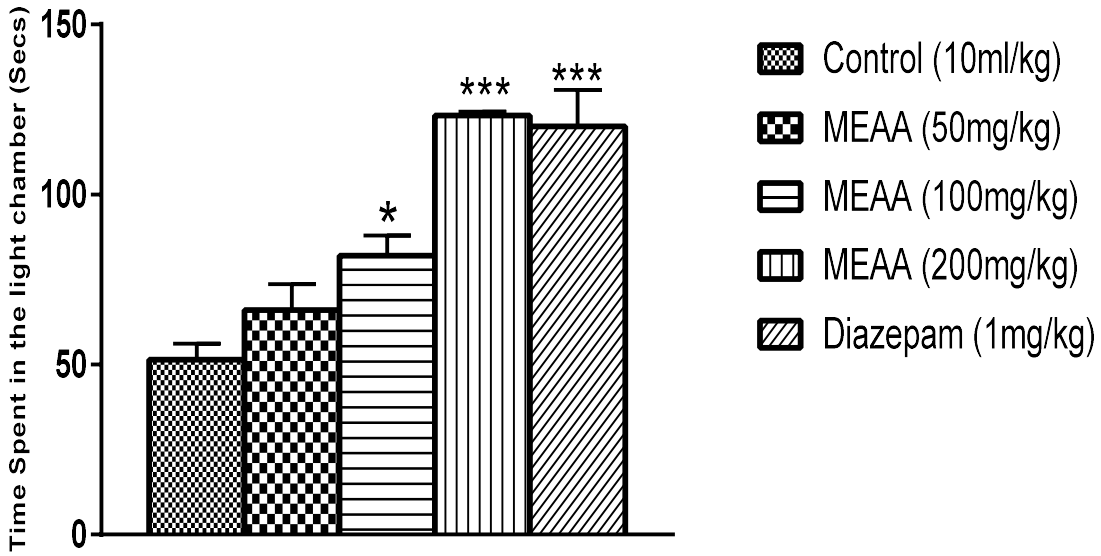

Fig 4.6: Behavioural Potentials of MEAA as well as Diazepam on Anxiety Status of mice in LDT. Results presented as mean ± SEM (n=6). Data analyzed by One-way ANOVA, followed by Newman Keuls' post hoc test. *$p<0.05$, ***$p<0.001$ compare to control.

Effect of Methanol Extract of *Artocarpus altilis* onImmobility Time in Forced Swimming Test

Immobility time in forced swimming test was used to evaluate the potentials ofmethanol extract of *Artocarpus altilis*(100 and 200 mg/kg)and Imipramine (10 mg/kg). The study showed significant ($p<0.05$) decrease in the treatment groupswhen compared with control (Fig.4.7).

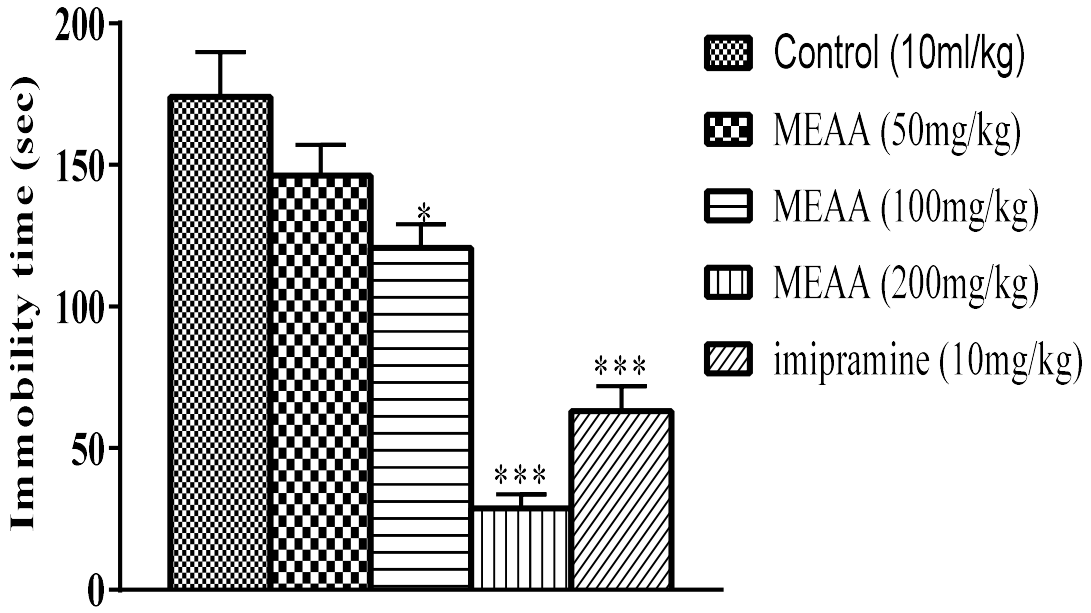

Fig 4.7:Behavioural Potentials of MEAA as well as Imipramine on depression Status of mice in FST model. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test.*p<0.05, ***p<0.001 compared with control.

Effect of Methanol Extract of *Artocarpus altilis* on Climbing Time in Forced Swimming Test

Methanol extract of *Artocarpus altilis*(200 mg/kg) and Imipramine (10 mg/kg)were shown to exhibit significant ($p<0.05$)increasein climbing time in forced swimming test when compared with control (Fig. 4.8)

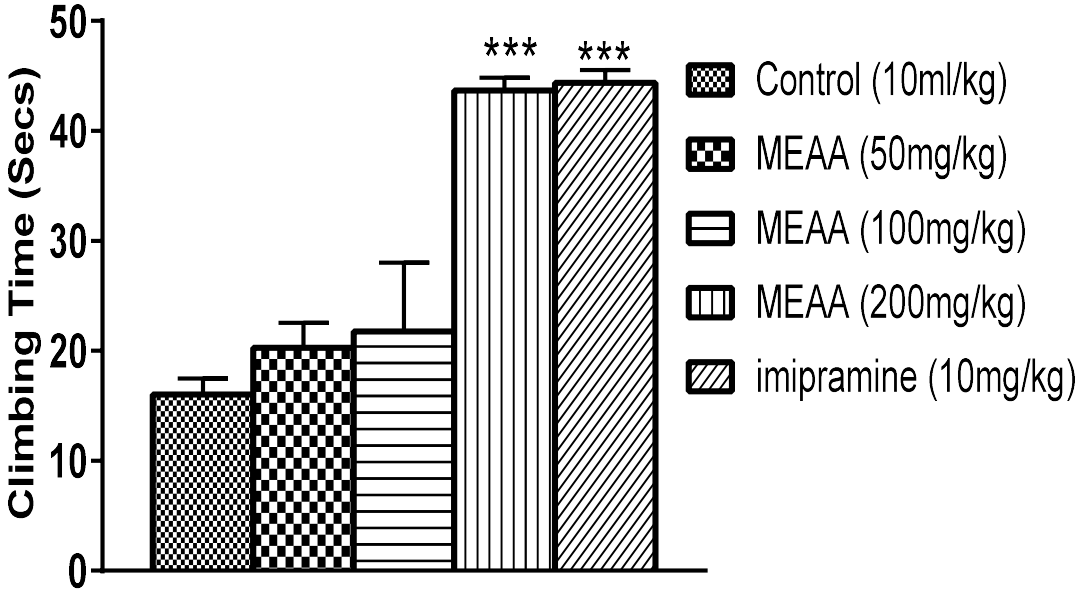

Fig 4.8: Behavioural Potentials of MEAA as well as Imipramine on depression Status of mice in FST. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test.*$p<0.05$, ***$p<0.001$ compared with control.

Effect of Methanol Extract of *Artocarpus altilis* on Immobility time in Tail Suspension Test

As presented in Figure 4.9,methanol extract of *Artocarpus altilis*(100 and 200 mg/kg)and Imipramine (10 mg/kg) wereshown to have significant ($p<0.05$)decrease inimmobility timein tail suspensiontest when compared with control.

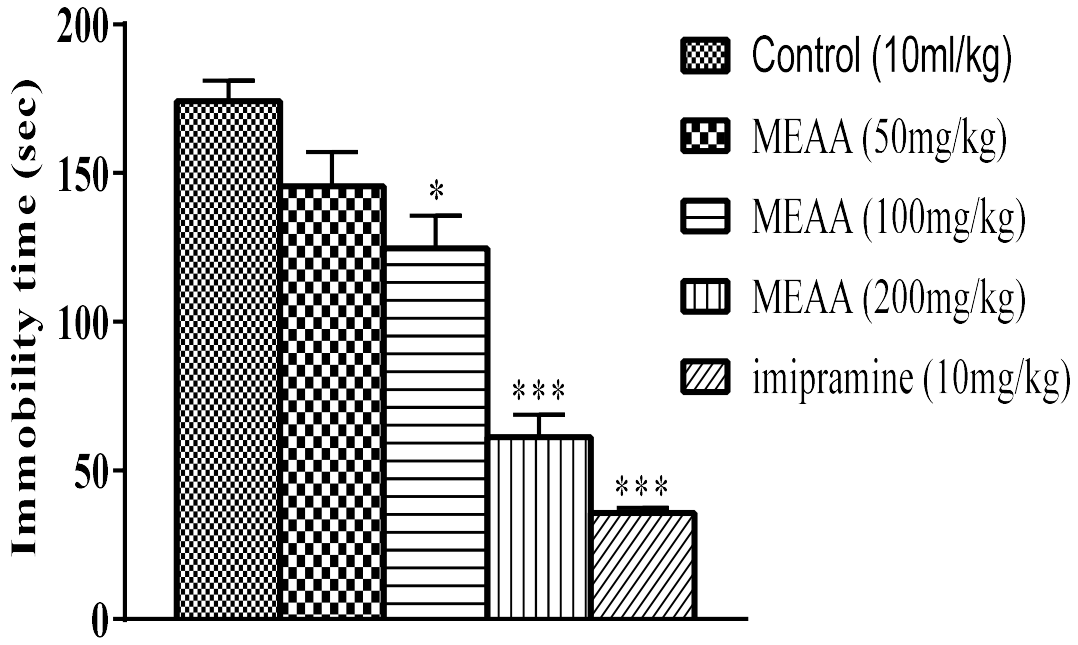

Fig 4.9: Behavioural Potentials of MEAA as well as Imipramine on depression Status of mice in TST Model. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test.*$p<0.05$, ***$p<0.001$ compared with control.

Effects of Methanol Extract of *Artocarpus altilis*on Number of Line Crossing in Open Field Test

Methanol extract of *Artocarpus altilis*(50, 100 and 200 mg/kg)and Imipramine (10 mg/kg)showed significant ($p<0.05$)decrease in number of line crossingwhen compared with control (Fig. 4.10).

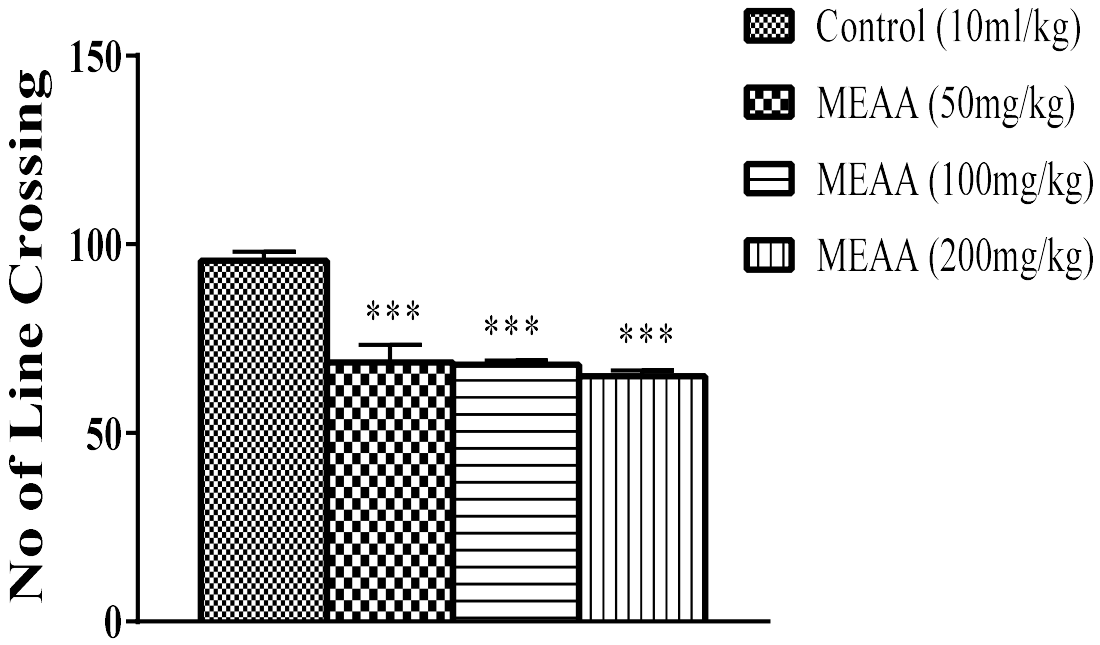

Fig 4.10:Behavioural Potentials of MEAA on locomotory Status of mice in OFT model. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test.*$p<0.05$, ***$p<0.001$ compared with control.

Involvement of Monoaminergic Systems on Immobility in Antidepressant-Like Potentials of Methanol Extract of *Artocarpus altilis* in Tail Suspension Test

As presented in Figure 4.11, prazosin (1mg/kg i.p an alpha-1-adrenergic receptor antagonist), sulpiride (50mg/kg i.p.,a dopamine D_2 receptor blocker) and metagoline (4mg/kg i.p., a $5HT_2$ receptor antagonist) showed significant ($p<0.05$) increase in the immobility time when compared to MEAA (200 mg/kg), whichoriginally decreased the immobility time.

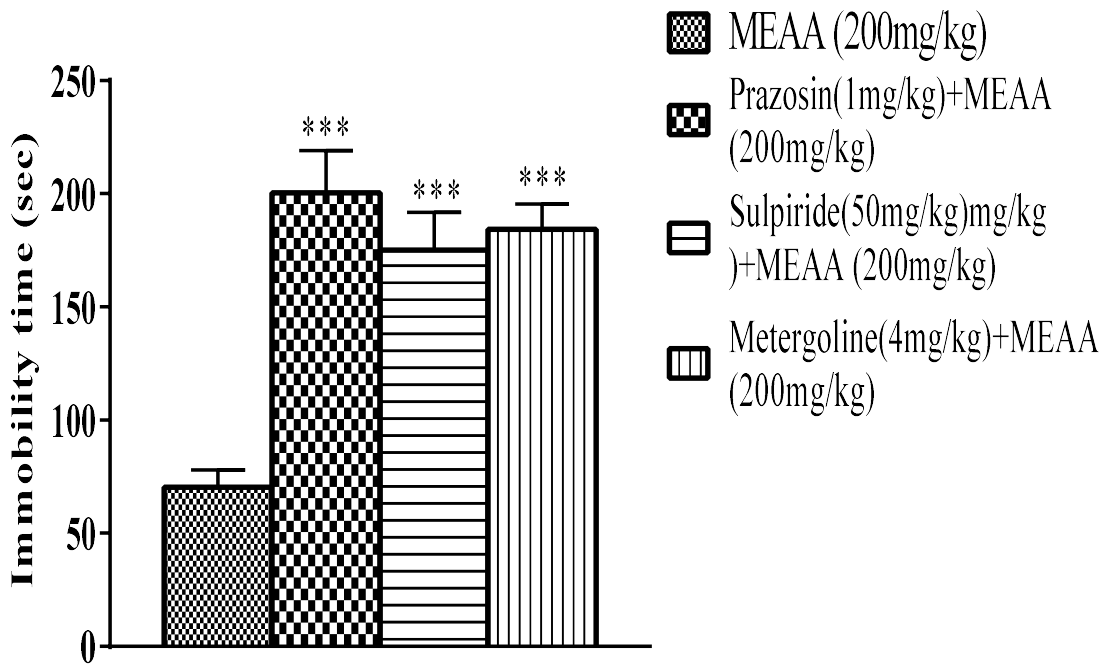

Fig 4.11: Effect of prazosin, sulpiride or metergoline on anti-depressant-like activity ofMEAA in mice. Each bar represents the mean ± SEM (n=6). Data analyzed by One-way ANOVA, before subjection to Newman Keuls' post hoc test. ***$p<0.001$ compared to MEAA.

Effects of Methanol Extract of *Artocarpus altilis* on Immobility Time in LPS-Induced Depression-like Behaviour in FST

As shown in Figure 4.12, methanol extract of *Artocarpus altilis* in combination with LPS (50 mg/kg + LPS 0.5mg/kg, 100 mg/kg + LPS 0.5 mg/kg, 200 mg/kg+ LPS 0.5 mg/kg) and imipramine in combination with LPS (IMI 10 mg/kg + LPS 0.5 mg/kg) showed significant ($p<0.05$)difference in the immobility timewhencompared with LPS and control groups.

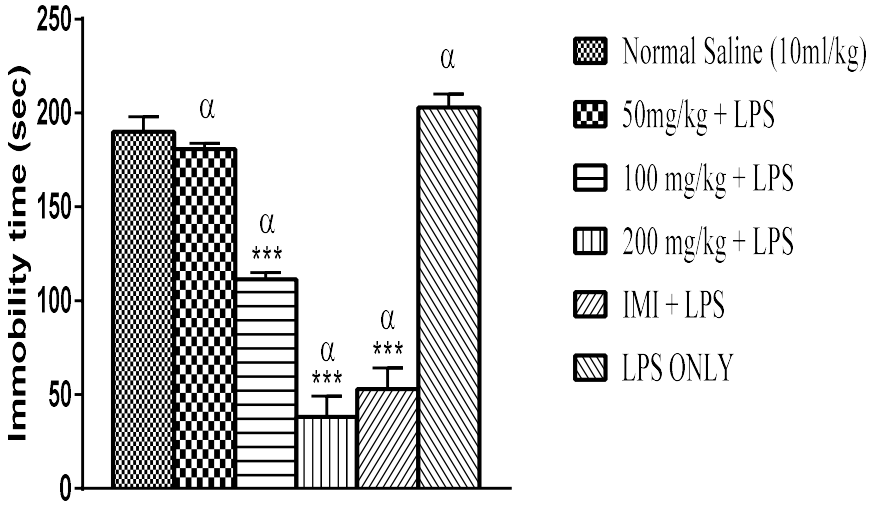

Fig 4.12: Behavioural potentials of MEAA *on* LPS-Induced Depression-like Immobility status of mice in FST Model. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test. ***$p<0.001$ compared with LPS only; $^{\alpha}p<0.001$ compared with Control.

Effects of MEAA on Number of line crossing in LPS induced Depression-like Behaviour in OFT.

Methanol extract of *Artocarpus altilis*in combination with LPS (50 mg/kg + LPS 0.5mg/kg, 100 mg/kg +LPS 0.5 mg/kg, 200 mg/kg +LPS 0.5 mg/kg) and Imipramine in combination with LPS (IMI10 mg/kg +LPS 0.5 mg/kg) showed significant ($p<0.05$)difference in the number of line crossing when compared with LPS and control groups (Fig.4.13).

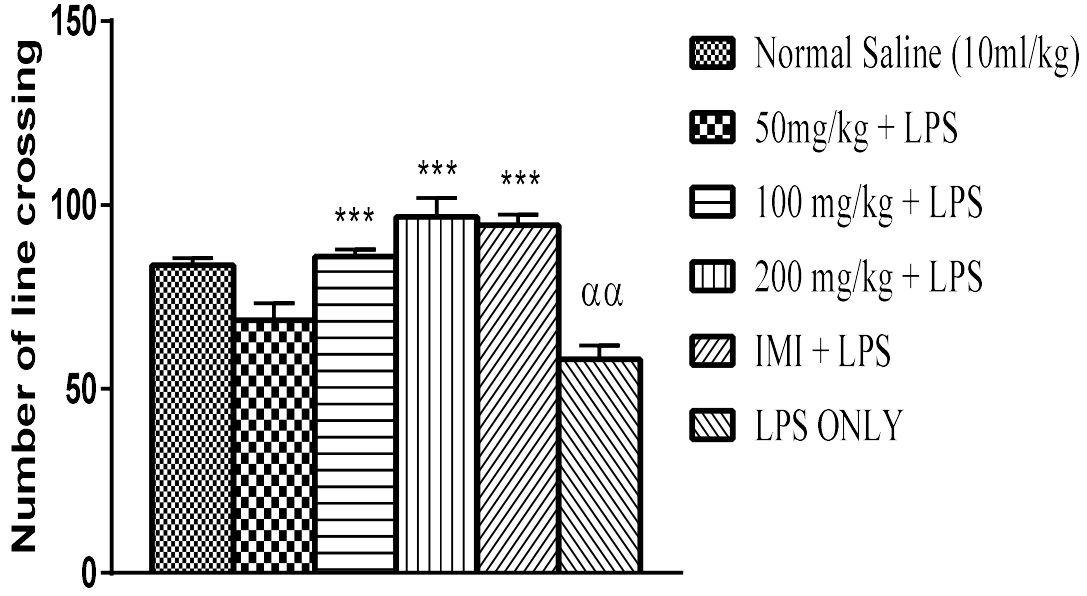

Fig 4.13**:** Behavioural potentials of MEAA *on* LPS Induced Depression-like Locomotory status of mice in OFT. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test. ***$p<0.001$; compared with LPS Only. $^{\alpha\alpha}p<0.01$ with control.

Effect of Methanol Extract of *Artocarpus altilis* in mice on LPS Induced Depression-like Behaviour in Protein Level

Methanol Extract of *Artocarpus altilis*showed no significant($p > 0.05$) difference on protein level in LPS treated mice when compared with LPS and control groups as shown inFigure 4.14.

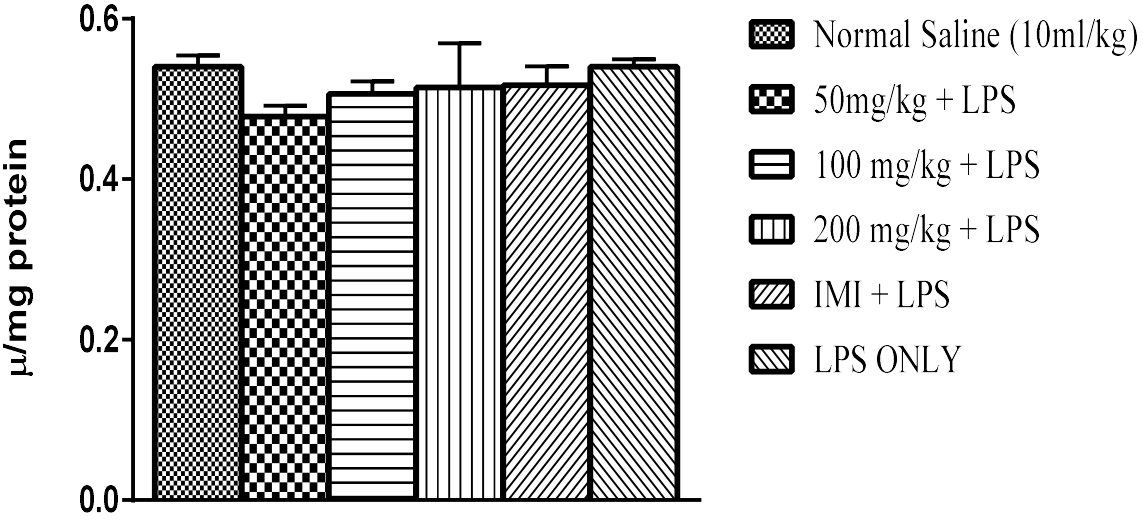

Fig 4.14: Determination of Protein Levels in the Brain of Animals Pre-treated with MEAA as well as Imipramine before LPS. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test. Not significant when compared with both Control and LPS Only.

Effects of Methanol Extract of *Artocarpus altilis* on LPS Induced Depression-like Behaviour in Reduced Glutathione (GSH) Level

In Figure 4.15,methanol extract of *Artocarpus altilis*in combination with LPS (50 mg/kg + LPS 0.5mg/kg, 100 mg/kg +LPS 0.5 mg/kg, 200 mg/kg +LPS 0.5 mg/kg) and Imipramine in combination with LPS (IMI 10 mg/kg +LPS 0.5 mg/kg) showed significant ($p<0.05$) difference in Reduced Glutathione (GSH) Level when compared with LPS and control.

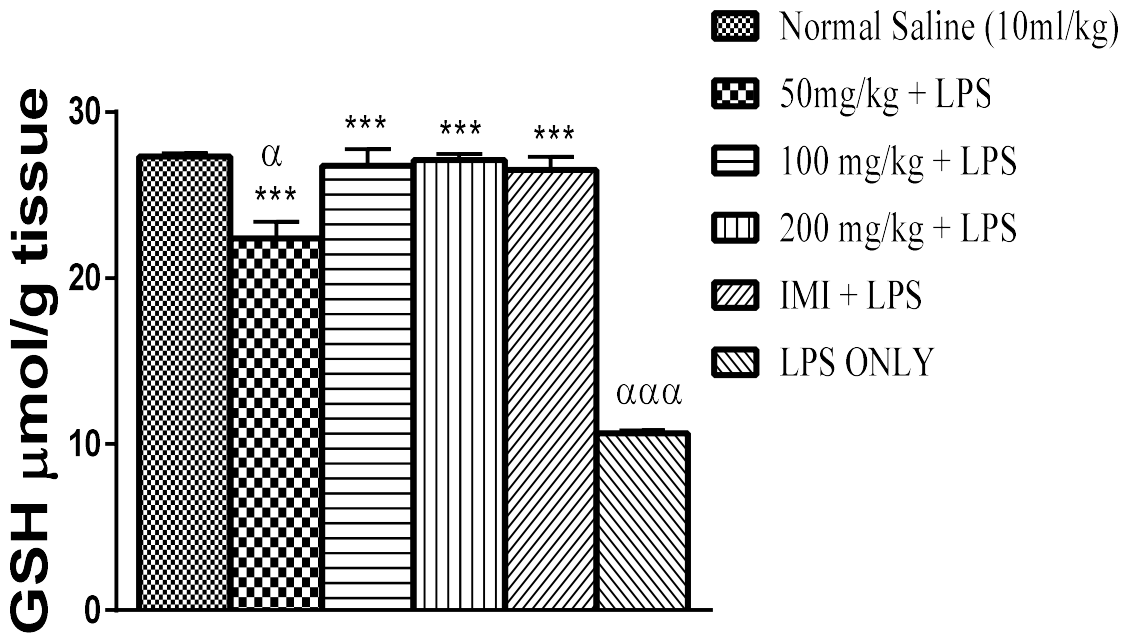

Fig 4.15: Effect of MEAAas well as Imipramine on the brain tissue level of Reduced Glutathione (GSH) (µmol/g tissue) content in the Brain of Mice in lipopolysaccharide- Pre-treated mice. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test. ***$p<0.001$ compared with LPS only; $^{\alpha}p<0.05$, $^{\alpha\alpha\alpha}p<0.001$ compared with Control.

Effects of Methanol Extract of *Artocarpus altilis* on LPS Induced Depression-like Behaviour in Superoxide Dismutase (SOD) Level

As presented in Figure 4.15,methanol extract of *Artocarpus altilis*in combination with LPS (50 mg/kg + LPS 0.5mg/kg, 100 mg/kg +LPS 0.5 mg/kg, 200 mg/kg +LPS 0.5 mg/kg) and imipramine in combination with LPS (IMI 10 mg/kg +LPS 0.5 mg/kg) showed significant ($p<0.05$) difference in SOD Level when compared with LPS and control.

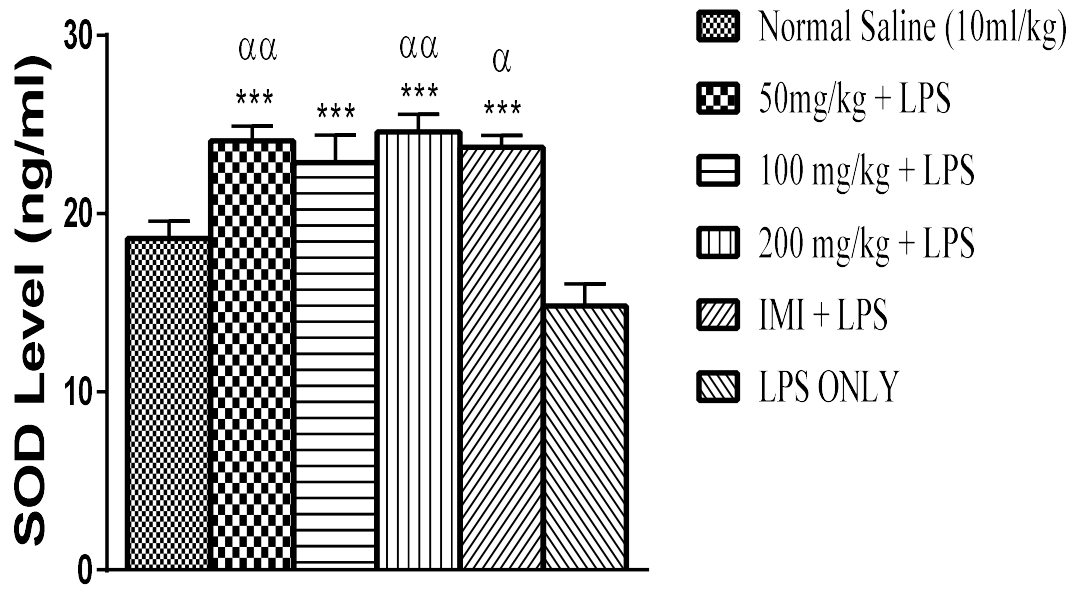

Fig 4.16: Determination of SOD Levels in the Brain of Mice Pre-treated with MEAAas well as Imipramine before LPS. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test. ***$p<0.001$ compared with LPS only; $^{\alpha}p<0.05$, $^{\alpha\alpha}p<0.01$ compared with Control.

Effects of Methanol Extract of *Artocarpus altilis* on LPS Induced Depression-like Behaviour in Nitric Oxide (NO) Level

As presented in Figure 4.17,methanol extract of *Artocarpus altilis*in combination with LPS (50 mg/kg + LPS 0.5mg/kg, 100 mg/kg +LPS 0.5 mg/kg, 200 mg/kg +LPS 0.5 mg/kg) and imipramine in combination with LPS (IMI 10 mg/kg +LPS 0.5 mg/kg) showed significant ($p<0.05$) difference in Nitric Oxide Level when compared with LPS and control.

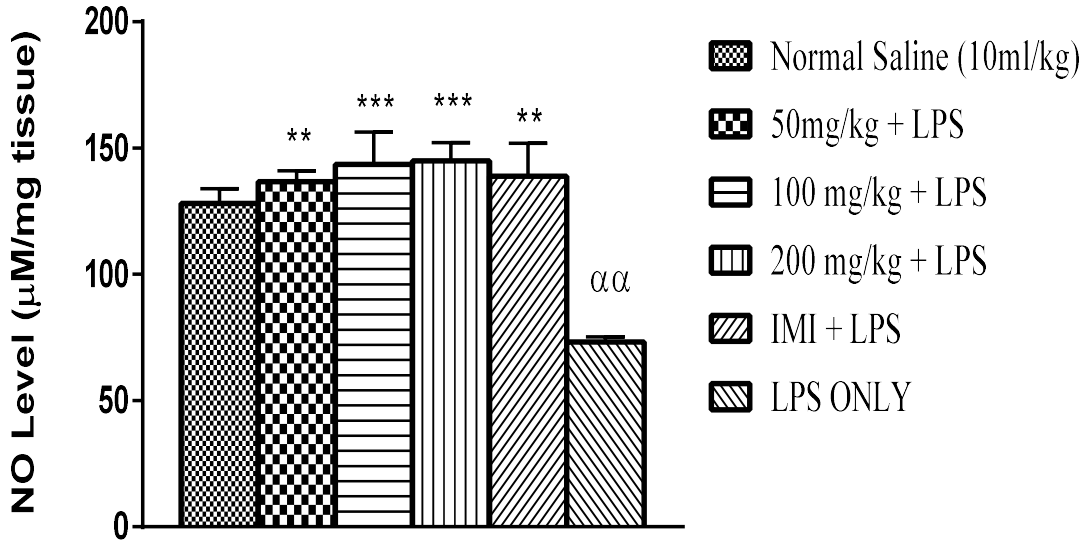

Fig 4.17: Determination of Nitrite Levels in the Brain of Animals Pre-treated with MEAAas well as Imipramine before LPS. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test. **p<0.01, ***p<0.001 compared with LPS only; $^{\alpha\alpha}$p<0.01 compared with Control.

Effects of Methanol Extract of *Artocarpus altilis* on LPS Induced Depression-like Behaviour in Thiobarbituric Acid Reactive Species (TBARS)Level

Methanol extract of *Artocarpus altilis*in combination with LPS (50 mg/kg + LPS 0.5mg/kg, 100 mg/kg +LPS 0.5 mg/kg, 200 mg/kg +LPS 0.5 mg/kg) and imipramine in combination with LPS (IMI 10 mg/kg +LPS 0.5 mg/kg) showed significant ($p<0.05$) difference in TBARS levelwhen compared with LPS and control (Fig. 4.18).

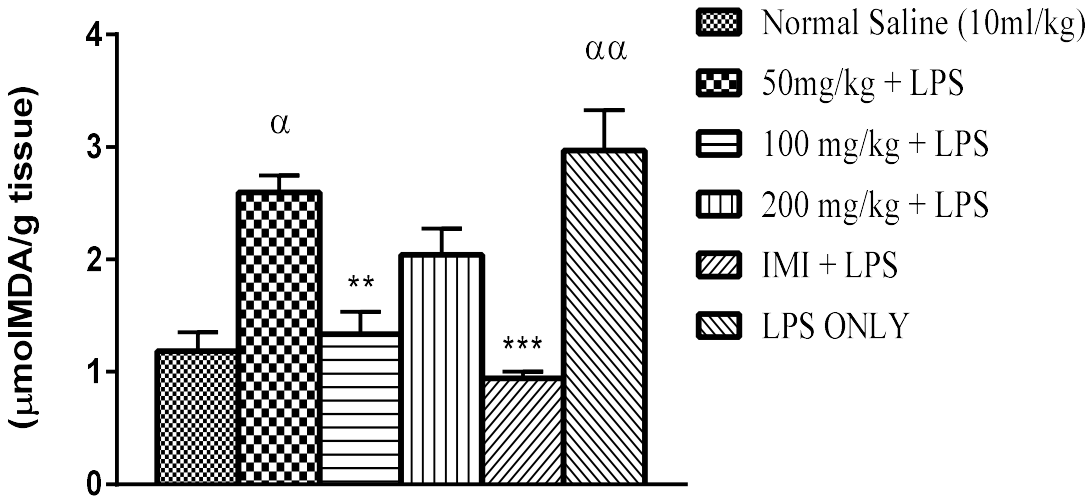

Fig 4.18: Thiobarbituric acid reactive substances (TBARS) levels in the Brain of mice Pre-treated with MEAA as well as imipramine before LPS. Results presented as mean ± SEM (n=6). Data analyzed by Oneway ANOVA, followed by Newman Keuls' post hoc test. **p<0.01, ***p<0.001 compared with LPS only; $^{\alpha}$p<0.05, $^{\alpha\alpha}$p<0.01 compared with Control.

CHAPTER FIVE

Discussion and conclusion

This research work was carried out to investigate the effects of *Artocarpus altilis*on depression and anxietyin mice and the possible mechanism of action by investigating the involvement of serotonergic, noradrenergic and doperminergic systems.

Increased immobility duration, which is the time a test animal in FST is immobile (floating) after a period of struggling (swimming), have beenreported by Salome *et al.,* (2002) as well asKalueff*et al.,* (2004) toindicatedepression. In another terms, immobility signifies cessation of perseverance in behaviour targeted at running away. Significantdecrease in immobility duration following various doses of MEAA (50, 100, 200mg/kg) administration and the standard drug (Imipramine) was observed when compared with control group. Hence, immobility occurrence separates the animal's effective methods of managing thetraumatic events (Lucki, 2001). This result suggests that MEAApossess anti-depressant- like effect in mice.

Furthermore, 200mg/kg MEAA treatment group significantly increased the climbing time (duration) which serves as a compensatorymeans for the decreased immobility earlier reported, although, the group pretreated with imipramine did not have significant effect.Barros and Ferigolo, (1998), also reported similar findings, which stated that Imipramine-like drugs does not affect climbing time in forced swimming test. Immobility might remain comparable to the scientific findings stating that patients with depressed moodare frequently deficient incontinued outflow of energy replicated in a marked psychomotor damage(Weingartner and Silberman, 1982). Immobility duration is applied in themeasurement of the overall antidepressantor depression-like consequence of a drug (Taiwo *et al.,* 2012). Hence, MEAA can be said to exhibit anti-depressant-like potentials.

The monoamine hypothesis of depression suggests that the reduction in serotonin, norepinephrine, and/or dopamine levelwithin central nervous system (CNS)is the pathophysiologicalbasisof depression (Coppen, 1967). This study shows that MEAA at 200 mg/kg, reduced immobility in laboratory mice, which suggests antidepressant activity.However, theanti-immobility effect of the extract was attenuated bypretreatment of mice with metergoline, an antagonist of non-selective $5HT_2$ receptor. This finding suggests that the antidepressant effect of the extract might be mediatedby interactions with serotonergic system. Pre-treatment with prazosin (1mg/kg), antagonist of alpha (α) 1 adrenoceptor also overturned the decreased immobilityproduced by MEAA in the TST. This implies that MEAA mightbe interacting with $alpha_1$-adrenergic receptors. Dopaminergic system has also been linked withmood regulation.There is a propositionthat an increase in dopamine neurotransmission maycounter the anhedonic effect, being a prominent symptom of depression(D'Aquila *et al*., 2000). This study observed that sulpiride, a dopaminergic (D_2) antagonist reversed the reduced immobility produced by MEAAin TST. This may perhaps implies thatdopaminergic system is involvedin the anti-depressant-like action of MEAA.

Elevated plus Maze, Elevated zero Maze and LDTparadigms were carried out toassess anxiety-related behavioural patterns in rodents (Salim *et. al.,* 2010b). These three interconnected assays particularly target towards measuring anxiety- linked defence behaviours similarly described (Approach-avoidance conflict tests) (Griebel and Holmes, 2013).Investigation of Anxiety-related behaviours depend upon connections amongst emotional behavioural pattern of humansas well as behavioural pattern of other creatures (Darwin, 1998), particularly mice and rats (Prut and Belzung, 2003). EPM andEZM models depend upon mice's dislike for open areas termed as thigmotaxis. Increased number of interval mice spends in the close arms in course of a5-minutesduration is suggestive of excessive anxiety-related behaviour. Number of time spent in open arms ofboth EPM and

EZM by the mice treated with 200mg/kg of MEAA ($p<0.05$) was significantly higher than the control. These increases in the numbers of entryinto the open arms in both EPM and EZM compared to respective control groups were quite similar to standard drug DZP. This depicts anxiolytic-like behaviour as stated by a couple of researchers (Pellow and Sandra, 1986; Budzynska*et al.,* 2013; Mansouri *et al*., 2014).

The amount of time mice spendswithin light chamber and number of times it enteredlight chamber exploited askey to assess anxiety-related conditionsin mice (Suzuki *et al.,* 2007). The result revealed that oral administration of MEAA (200mg/kg) as well as DZP (1mg/kg) respectively, caused significant ($p<0.05$) increase in the number of entries into the light chamber and time spent in the light chamber than in the dark chamber of the LD Board. This suggests an anxiolytic-like activity.Open field tests carried out on rodents postulated that decreased general locomotion (line crossing) is a sign of depression as amount (number) of line crossing is considered a quantity in locomotor activity (Andrade *et al.,* 2003). Oral administration of MEAA caused significant ($p<0.05$) decrease in the number of line crossing. The decreased number of line crossing could be said to be sedative (calming effects), which is associated withanxiolytic drugs.

It has been observed that the LPS i.pshot, dosage0.5 mg/kg caused significant upsurge in duration of immobility in FST model relative to control, whereas MEAA (100mg/kg and 200mg/kg) as well as IMP (10mg/kg) reduced immobility. MEAA, in the same way as imipramine,considerably reduced time of immobility induced by LPS, thus signifying an anti-depressant-like action. However, MEAA as well as imipramine administration did not affect the locomotor activity. Conversely, animals pre-treated with LPS showed decreased locomotor activity in comparison with control.

In this study, MEAA and imipramine pre-treatment restored LPS-induced decrease in nitrite concentration, which happens to be a measure of nitric oxidetissue concentrations. Thus, an earlier account point to the fact that non-selective nitric oxide synthase antagonist (NG-nitro-L-arginine (L-NAME) elevated sickness behaviour induced by LPS in rats, implying that endogenous nitric oxide is not associated with sickness behaviour induced by LPS, rather may possess defensive task (Ribeiro *et al.,* 2013). Hence, LPS may be exerting its effect by potentiating the action of NG-nitro-L-arginine (L-NAME) which regulates the endogenous NO concentration. Hypothalamic pituitary adrenal (HPA) axis inhibition observed within elevated cytokine concentration, also in neuropeptide secretion as seen in immune stimulation may be linked to reduction in nitrite concentration seen in this result (Uribe *et al.,* 1999; Jankord*et al.,* 2009).Recent findings suggest that reduction in level of nitrite/nitrate in the CSF observed in persons with mood conditions indicates a reduction in nitric oxide production (Gao *et al.,* 2012). However, depression-related behaviours often associated with surplus as well as scarcity of nitric oxide are facilitated through stimulation of cyclic adenosine monophosphate response element binding protein (CREB) (Hu *et al.,* 2012). Accordingly, it was observed that LPS administrationbrought about a reduction in GSH concentration. Importantly, both MEAA (except 50 mg/kg) and imipramine blocked and upturned such changes. MEAA and imipramine had comparable behavioural andbiochemicalproperties in this study, buttressing the fact that microglia stimulation might be the central pathophysiology of LPS-induced depression-related effects(Tynan *et al.,* 2012). In line with the findings, 0.4 ng/mL LPS shot produced a noticeable temporary plasma concentrations of cortisol upsurge, IL-1ra, IL-6, IL-10 as well as TNF-α with a mood reduction (Grigoleit*et al.,* 2011; Dunjic-Kostic*et al.,* 2012).

In this current investigation, the induction of depression-related behaviour in mice by intraperitoneal injection of LPS endotoxin (0.5 mg/kg)caused an elevated level of oxidative

stress biomarkers within the brain. Malondialdehyde, expressed as TBARS, (an indication of lipid peroxidation) is elevated within the brain tissue (Gutteridge, 1995). This action was linked to a noticeable as well as substantial reduction in concentration of reduced glutathione, a vital anti-oxidant defence mechanism.

CHAPTER SIX

SUMMARY AND RECOMMENDATIONS

6.1. SUMMARY

The results of this study suggest that methanol extract of *Artocarpus altilis*possesses anti-depressant and anxiolytic properties in mice supporting its use in traditional medicine. The anti-depressant-like activity of methanol extract of *Artocarpus altilis* may be related to inhibition of oxidative stress and enhancement of momoaminergic activity.

6.2. RECOMMENDATION FOR FURTHER STUDIES

Further studies involving evaluation of the effect of methanol extract of *Artocarpus altilis*on biomarkers of neuro-inflammation in LPS model of depression and its safety profile should be carried out. The determination of the active principle(s) responsible for its activity is also recommended for future studies.

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APPENDIX

Effects of MEAA on Sum of Entrances into the opened arms in E.P.M

Control	MEAA	MEAA	MEAA	Diazepam
10ml/kg	50mg/kg	100mg/kg	200mg/kg	1mg/kg
3	4	3	4	11
2	4	3	4	11
4	2	3	5	15
2	2	4	6	14
2	3	2	5	
2			6	

Effects of MEAA onPeriod Expended in the opened arms (Secs) in E.P.M

	MEAA	MEAA	MEAA	Diazepam
control	50mg/kg	100mg/kg	200mg/kg	1mg/kg
16	28	27	83	127
28	22	25	45	120
30	28	22	61	138
12				151
				139

Effects of MEAA on Sum of Entrances through opened arms in E.Z.M

Control	MEAA	MEAA	MEAA	Diazepam
10ml/kg	50mg/kg	100mg/kg	200mg/kg	1mg/kg
5	5	5	11	19
4	2	4	10	25
7	4	4	10	18
	2	5		

Effects of MEAA on Period Expended in the opened arms (Secs) in E.Z.M

Control	MEAA	MEAA	MEAA	Diazepam
10ml/kg	50mg/kg	100mg/kg	200mg/kg	1mg/kg
54	49	39	62	100
54	64	88	97	90
53	95	32	53	66
		88	86	103
		88		

Effect of MEAA on Number of Entries into the light chamber in L/D Transition Box

Control	MEAA	MEAA	MEAA	Diazepam
10ml/kg	50mg/kg	100mg/kg	200mg/kg	1mg/kg
4	6	5	8	8
4	5	6	7	8
5	4	5	7	8
5	7	4	8	10
6			8	9

Impact of MEAA on Period Expended on light chamber (Secs) in L/D Transition Box

Control 10ml/kg	MEAA 50mg/kg	MEAA 100mg/kg	MEAA 200mg/kg	Diazepam 1mg/kg
41	50	80	120	121
43	62	90	123	128
53	65	86	125	90
68	87	94	125	141
52		60		

Effect of MEAA on Immobility time (sec) in FST

Control	MEAA (50mg/kg)	MEAA (100mg/kg)	MEAA (200mg/kg)	imipramine (60mg/kg)
204	143	107	46	58
196	177	144	19	88
136	126	121	20	60
160	139	111	28	46
			31	

Effect of MEAA on Climbing Time (Secs) in FST

Control	MEAA (50mg/kg)	MEAA (100mg/kg)	MEAA (200mg/kg)	imipramine (60mg/kg)
13	26	27	42	42
16	22	12	46	48
15	17	11	43	46
20	16	37		44
				42

Effect of MEAA on Immobility time (sec) in TST

Control	MEAA (50mg/kg)	MEAA (100mg/kg)	MEAA (200mg/kg)	imipramine (60mg/kg)
157	167	108	78	35
165	135	136	70	39
169	162	105	48	33
195	118	150	49	
185				

Effect of MEAA on No of Line Crossing in OFT

Control 10ml/kg	MEAA 50mg/kg	MEAA 100mg/kg	MEAA 200mg/kg
91	68	70	67
97	61	68	66
99	77	66	62

Effects of MEAA on Mechanism of action using Tail Suspension Test

ED MEAA (200mg/kg)	Prazosin+ED MEAA	Sulpiride+ED MEAA	Metergoline+ED MEAA
78	262	174	179
95	214	224	134
70	190	184	215
82	123	130	199
48	216	127	194
49	198	212	185

Impact of MEAA on depressive-behaviour induced by LPS in F.S.T

Normal Saline	50mg/kg + LPS	100 mg/kg + LPS	200 mg/kg + LPS	IMI + LPS	LPS ONLY
209	180	103	78	58	189
189	179	120	46	88	215
184	183	115	19	21	222
164	190	117	20	60	185
204	173	103	28	38	204

Impact of MEAA on depressive-behaviour induced by LPS in O.F.T

Normal Saline	50mg/kg + LPS	100 mg/kg + LPS	200 mg/kg + LPS	IMI + LPS	LPS ONLY
86	76	90	100	90	65
80	60	81	87	89	57
85	70	88	110	100	52
		85	90	99	

Impact of MEAA on depressive-behaviour induced by LPS in Protein Level

Normal Saline	50mg/kg + LPS	100 mg/kg + LPS	200 mg/kg + LPS	IMI + LPS	LPS ONLY
0.580442	0.429022	0.523659	0.66877	0.498423	0.529968
0.529968	0.492114	0.460568	0.410095	0.59306	0.51735
0.529968	0.473186	0.51735	0.473186	0.51735	0.561514
0.498423	0.485804	0.523659	0.504732	0.529968	0.561514
0.561514	0.511041			0.44795	0.529968

Impact of MEAA on depressive-behaviour induced by LPS in GSH Level

Normal Saline	50mg/kg + LPS	100 mg/kg + LPS	200 mg/kg + LPS	IMI + LPS	LPS ONLY
27.658434	23.32548	27.80442	27.484392	27.09648	10.674321
26.894456	23.45	27.73579	26.359231	27.52647	10.954321
27.563871	20.3582	24.75	27.482413	24.88	10.337346
27.232345					

Impact of MEAA on depressive-behaviour induced by LPS in SOD Level

Normal Saline	50mg/kg + LPS	100 mg/kg + LPS	200 mg/kg + LPS	IMI + LPS	LPS ONLY
22.2998	24.01513	26.04054	21.13334	21.88721	17.80899
17.15369	26.19837	19.0964	25.8276	25.73054	12.00759
17.22825	24.97576	21.71232	24.38459	24.35337	13.72296
17.80899	21.18984	24.62	27.01704	22.99323	15.74
18.62	24.09		24.59	23.74	

Impact of MEAA on depressive-behaviour induced by LPS in Nitric Oxide level

Normal Saline	50mg/kg + LPS	100 mg/kg + LPS	200 mg/kg + LPS	IMI + LPS	LPS ONLY
143.1034	130.3	158.6207	166.5517	138.9655	70
114.4828	132.069	167.2414	134.1379	116.2	76.8554376
117.5862	149.3103	109.3103	140.456	161.3793	72.76
125.1	135.5172	138.9	138.59		
140.34224					

Impact of MEAA on depressive-behaviour induced by LPS in Thiobarbituric acid reactive species (TBARS) level

Normal Saline	50mg/kg + LPS	100 mg/kg + LPS	200 mg/kg + LPS	IMI + LPS	LPS ONLY
1.460537	2.348745	0.964	1.804404	0.887415	2.328868
0.887415	2.556456	1.657633	2.509583	0.879951	3.854632
1.207244	2.879839	1.378591	1.809645	1.05881	2.438594
					3.256327