EVALUATION OF ANTIPSYCHOTIC EFFECTS OF METHYL JASMONATE IN MICE

BY

Olajide Saheed, ANNAFI

MBBS (Ilorin); M.Sc Pharmacology and Therapeutics (U.I)

(MATRIC No. 167574)

A DISSERTATION SUBMITTED TO THE DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS, FACULTY OF BASIC MEDICAL SCIENCES, UNIVERSITY OF IBADAN IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF

MASTER OF PHILOSOPHY (PHARMACOLOGY)

OF THE

UNIVERSITY OF IBADAN, NIGERIA

JANUARY, 2017

CERTIFICATION

I certify that this work is an original research carried out by Dr. Olajide Saheed ANNAFI of the Department of Pharmacology and Therapeutics, College of Medicine, University of Ibadan, Ibadan, Nigeria.

SUPERVISOR **Date**

Dr. Solomon Umukoro

B.Sc. (Lagos), M.Sc. (Lagos), Ph.D. (Lagos).

Department of Pharmacology and Therapeutics,

University of Ibadan.

DEDICATION

This work is dedicated

To

Our Lord Jesus and my parents

ACKNOWLEDGEMENTS

I really appreciate God (Father, Son, and Holy Spirit) for unlimited grace and love showered continually on me all this while.

I am very grateful to my parents for their unrelented care and concern about me. I am thankful for their moral and physical support.

It is extremely important to show my profound gratitude to my supervisor, Dr Solomon Umukoro, for his unswerving effort in equipping me with necessary knowledge and skills needed in research. His thorough and diligent supervision has imbibed in me the practical knowledge necessary for research methodology.

I thank all my lecturers: Prof. E. O. Iwalewa (the H. O. D.), Prof. Akintunde Sowunmi, Prof. Catherine O. Falade, Prof. Ayo Adeagbo, Prof. G. Ademowo, Prof. Fehintola F. A., Prof. Grace O. Gbotosho, Dr. D. A. Adedapo and Dr. Oyindamola O. Abiodun. I also sincerely appreciate Dr Adegbuyi O. Aderibigbe for his assistance towards the success of my research. Also to Dr. Abayomi M. Ajayi, Dr. Bakre G. A. and other members of the academic staff of the Department.

My appreciation also goes to Dr A.T. Eduviere, Dr. I. Adeoluwa, Mr. A. Olatunde, Mr.O. Adegoke, Mr. A. Akintoye, and Mr.O. Liasu for kind assistance all through my work.

I really appreciate my wife Mrs. Mary Annafi for her support and understanding. Thank you, really appreciate you.

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ABSTRACT

Psychosis is a chronic neurological disorder that impairs the quality of life of the patients and remains a major health challenge worldwide. Current drugs used to manage psychosis are expensive and only provide symptomatic relief without altering the underlying pathological derangement. Methyl jasmonate (MJ) is a bioactive compound known to have beneficial effects against a wide range of neurological disorders. However, its usefulness in treatment of psychosis has not been scientifically proven. Thus, this study was undertaken to investigate the effects of MJ on psychosis in animal models.

Fifty male Swiss mice (23.5±1.5 g) were assigned to 10 groups to evaluate acute antipsychotic-like effect of MJ on bromocriptine or ketamine-induced stereotypy. Groups 1-5 received 1% ethanol (vehicle, 10 mL/kg, i.p.), MJ (25, 50, 100 mg/kg, i.p.) and haloperidol (1 mg/kg, p.o.) 60 minutes prior to bromocriptine (5 mg/kg, p.o.) treatment, while groups 6-10 received vehicle (10 mL/kg, i.p.), MJ (25,50,100 mg/kg, i.p.) and risperidone (0.5 mg/kg, p.o.) 60 minutes prior to ketamine injection. Thereafter, each mouse was placed independently in an observation chamber (20 cm × 20 cm × 23 cm) and stereotyped behaviours were observed for 2 min at 10, 15, 30, 45 and 60 min after bromocriptine or ketamine injection. Another 36 mice (n = 6) were also allotted into treatment groups. Group 1 received vehicle (10 mL/kg, i.p.) while groups 2-6 were treated with ketamine (20 mg/kg, i.p.) once daily, for 14 days. Then, from 8th to 14th day, group 2 was treated with vehicle (10 mL/kg, i.p.) while Group 3-6 received MJ (25,50.100 mg/kg, i.p.) and risperidone (0.5 mg/kg) 60 minutes after ketamine injection. Hyper-locomotion was then measured as an index of psychotic-like behaviour using the open field chamber while memory was assessed using the Y-maze. Thereafter, whole brain samples were used to assay for malondialdehyde, reduced glutathione (GSH), catalase and Superoxide Dismutase (SOD) using spectrophotometric techniques. Histology of prefrontal cortex, hippocampus and substantial nigra were viewed in ketamine-treated mice and neuronal density was determined. Data were analysed using descriptive statistics and ANOVA at $\alpha_{0.05}$.

Methyl jasmonate (25, 50 and 100 mg/kg) significantly reduced stereotypy score (0.62±0.21, 0.24±0.10 and 0.06±0.04) relative to vehicle (1.84±0.15) and (0.50±0.08, 0.36±0.06, 0.26±0.07) relative to vehicle (1.52±0.10) induced by bromocriptine and ketamine, respectively. Methyl jasmonate significantly ameliorated ketamine-induced hyper-locomotion (74.67±4.70, 75.67±2.88, 78.00±4.16 s) compared to vehicle (185.0±3.63) and memory deficit (80.1±2.8, 71.2±2.9, 57.0±3.4 %) relative to vehicle (53.7±2.0%). Methyl jasmonate reduced malondialdehyde concentration (19.96±1.64, 22.84±1.16, 24.65±1.70 µmol/g tissue) relative to vehicle (33.72±2.28 µmol/g tissue) but increased GSH levels (47.43±2.22, 42.23±2.83, 37.26±1.84 µmol/g tissue) compared to vehicle (21.95± 2.69 µmol/g tissue). Methyl jasmonate also increased catalase level in the brain homogenate (86.63±4.65, 83.36±4.24, 76.06±3.22 units/mg protein) relative to vehicle (59.91±3.94 units/mg) and SOD (27.52±1.63, 24.41±1.49, 19.71±1.59 units/mg protein) compared with vehicle (13.08±1.33 units/mg protein), respectively. Brain histology revealed that MJ has protective property on neuronal cells compared to ketamine-treated mice.

Methyl jasmonate demonstrated antipsychotic property via activation of antioxidation pathway in Swiss mice.

Keywords: Methyl jasmonate, Memory enhancement, Cholinergic neurotransmission, Oxidative stress.

Word count: 492

ABBREVIATIONS

A ch	Acetylcholine
CNS	Central Nervous System
DA	Dopamine
EEG	Electroencephalogram
GAD	Glutamic acid decarboxylase
GSH	Glutathione
GST	Glutathione-s- transferase
HLP	Haloperidol
KET	Ketamine
MDA	Malondialdehyde
MJ	Methyljasmonate
RIS	Risperidone

CHAPTER ONE

1.0 INTRODUCTION

1.1 PSYCHOSIS

Psychosis is a form of mental illness characterized by abnormal behaviours with little or no touch with reality (Kreyenbuhl *et al*., 2009). It is characterized by multiple symptoms affecting thoughts, emotion, perception, and volition. It is a severe form of mental illness affecting the quality of life of the affected individuals. It has a huge untold burden on both the sufferer and the community at large. Hence, it constitutes a major financial burden and global health challenge requiring a continuous capital involvement (Picchioni, 2007).

Psychosis is a global health problem and studies have shown that about 450 million people across the globe have one form of brain or mental disorder. However, its distribution varies across the World from Country to Country (Kirkbride *et al*., 2006). The World Health Organization (WHO) showed that about 0.3- 0.7% of people demonstrate some features of psychosis at one point or the other in their life time (WHO, 2011). The prevalence of psychosis is 45.8% in Nigeria. The figures seemingly higher than between 25% and 38% from some countries of the World (Lasebikan *et al.*, 2012). However, this figure is known to be higher among Africans (Ewhrudjakpor, 2009). The age mostly affected is between 20 and 40 years with the peak at 28 to 34 years (Ewhrudjakpor, 2009). The global burden of this disease has been projected to increase to about 15% before the year 2020 (Ghodse, 2003).

Psychosis is a constellation of disorders with different aetiologies and behavioural manifestations; treatment of which depends on the type of disorder. Psychotic disorders could be mood disorders (depression, mania), schizophrenic psychosis, schizophreniform psychosis, schizoaffective disorders, schizotypal psychosis, substance-induced psychosis, dementia and paranoid personality (Malaspina *et al.*, 2013). Schizophrenic psychosis is the commonest. It is associated with the highest suffering and still the most costly to manage (Gold *et al*., 1991). Onset is commonly in the late adolescence years and characterized by delusions, catatonia, stereotyped behaviours and hallucinations (Baldessarini, 2001; Famous and Kindler, 2008). Patients with psychotic disorder are prone to depression, anxiety, suicidal tendencies, aggression,

victimization, poverty, substance abuse, cognitive impairment and increased health challenges (Mullen, 2006). The manifestations of psychotic disorders are commonly known as positive, negative and cognitive symptoms (Rollins *et al.*, 2010; Aline *et al.*, 2013). Cognitive dysfunction and negative symptoms are very important in disease progression, yet treatment compliants show limited improvement (Velligan *et al.*, 2008).

The aetiology of psychosis is often multifactorial; genetic, neuro-developmental and environmental factors (Lewis, 2000). It involves overlapping neurotransmitters dysfunctions and endocrine malfunctions. Biochemical hypothesis implicate dopaminergic system hyperactivity (Owens *et al.*, 2005). Some drugs are known to induce schizophrenia-like symptoms such as dopaminergic agents (apomorphine and amphetamine), glutamine receptor antagonists (ketamine and phencyclidine) and 5-hydroxyltryptamine agonists (Lysergic acid diethylamide). Hence, hypothesis around dopamine and serotonin excess are majorly implicated in the etiology of the disease (Davies, 1991; Baldessarini and Tarazi, 2001; Grayson *et al.*, 2005). However, hyperactivity of the dopaminergic system is the leading hypothesis of psychosis and this believe is based on alleviation of psychotic symptoms produced by antipsychotics like phenothiazines, thioxanthenes and butyrophenones (Owens *et al.*, 2005). However, factors such as persistent stress, substance abuse, obstetrics complications, environmental insults, genetic predisposition, and physical and emotional traumas have been implicated in the onset of psychosis. Out of all the risk factors, persistent stress is the highest cause of the disease (Ewhrudjiakpor, 2009; Lieberman, 1993).

Despite popularity of the scientific basis of psychosis, African's perception of the disease is totally different. African populace believed that the disease is the works of supernatural forces against mental well beings of the individuals and as such cure can only be obtained via traditional healing and religious practices; much of which processes are not only poorly understood but are also quite harmful and detestful to normal ethical rights and beliefs (Gureje, 2000).

The role of free radicals in the pathogenesis of schizophrenia has been proposed a long time ago (Hoffer *et al.*, 1954, Zhang *et al.*, 2010). Studies over the years have unraveled the role of impaired antioxidant defense system and oxidative stress as huge factors in schizophrenic

patients (Wood *et al*., 2009). Studies have shown alterations in the increased levels of oxidative stress and altered antioxidant defense mechanisms in schizophrenic brain (Woods *et al*., 2009).

Although pharmacological interventions have been the mainstay of treatment of the disease since the introduction of antipsychotic drugs in the 1950s, they have a number of limitations. These include high incidence of disabling side-effects, poor adherence to treatment and limited response of some patients to antipsychotic medications (Ray *et al*., 2009). The issues of high cost of drugs and adulteration are also sources of limitation in the pharmacotherapy of psychosis especially in developing countries (Kane, 2010). Above all, these drugs have failed to alter the course of the disease but only provide symptomatic relief (Davies, 1991). Furthermore, negative symptoms and cognitive deficits associated with the disease do not respond favourably well to the available antipsychotic drugs (Husa *et al*., 2014). Memory enhancement is a quality that is very important in antipsychotics so that patients can always have better recollection to use their drugs as a break off-drug state may be associated with a relapse or poor recovery state. The quality of life of patients suffering from the disease still remains very poor despite judicious use of the currently available antipsychotics. Thus, the need to search for new drugs especially agents with memory enhancing effect as alternative treatments for psychotic disorders still persist.

Methyl jasmonate (MJ) is a bioactive compound obtained from jasmine, a perennial climbing plant (*Jasminum grandiflorum*) that is well known for its sweet and highly scented flowers. Jasmine flower has been used in aromatherapy for depression, nervousness, tension, alertness and memory improvement (Kuroda *et al*., 2005). Methyl jasmonate, a cyclopentanoic compound, is one of the most well studied members of the jasmonate family, which has gained a global recognition as a potential agent for cancer chemotherapy (Cesari *et al*., 2014). The lack of toxicity to normal cells and the rapidity, by which it kills cancer cells, suggest that MJ may turn out to be a useful agent in cancer chemotherapy (Cesari *et al*., 2014; Belsito *et al*., 2012). Moreover, MJ has also gained international recognition as a plant hormone, which aids adaptation of plants to external stressors through the formation of proteinase inhibitor proteins; array of defensive chemical substances that protect plants against aversive conditions (Cesari *et al*., 2014; Bowles, 1990). However, it was reported that MJ exhibits sedative effect and

potentiates GABA-mediated inhibitory neurotransmission, which suggest its potential usefulness in neuropsychiatric disorders (Hossain *et. al.*, 2004). Moreover, previous studies have shown that intraperitoneal injection of MJ elicits antidepressant, anti-aggressive, adaptogenic/anti-stress and memory enhancing effects in experimental animals (Umukoro *et. al.*, 2011; Umukoro *et. al.*, 2012; Eduviere *et. al.*, 2015; Umukoro *et. al.*, 2016) suggesting potential benefits in the alleviation of some of the symptoms of psychosis.

Aim of the study

The aim of this study was to evaluate the antipsychotic activity of methyl jasmonate and the probable mechanisms underlying its action in animal models predictive of psychotic disorders.

Specific objectives

The specific objectives of the study include the following:

•Evaluate the effect of MJ on bromocriptine- and Ketamine-induced stereotypy in mice

•Evaluate the effect of MJ on chronic ketamine-induced cognitive dysfunction and hyperactivity in mice

•Biochemical assay of antioxidant system in mice exposed to chronic ketamine treatment

•Evaluation of the effect of MJ on the cytoarchitecture of the brain of mice chronically treated with ketamine

CHAPTER TWO

2.0 REVIEW OF RELATED LITERATURE

2.1.0. Basic Features of Psychosis

Psychosis is a severe form of mental illness whose constellation of symptoms is complex and quite challenging out of all the neuropsychiatric disorders. The most common being schizophrenia, a Greek word, which means "split personality". It is characterized by multiple disorientation and varying disorganized symptoms, which include delusions, hallucinations, thought disorder, inappropriate affect and impaired psychosocial functioning (Crimson, 2005). All these symptoms are often grouped together as positive, negative and cognitive symptoms. These diverse arrays of symptoms make it confusing whether psychosis is a single disease state or a syndromic state of diseases (Murray, 2007). Individuals with these diseases suffer in terms of quality of life, as well the family or healthcare givers in terms of huge and continuous monetary implication. Affected individuals are susceptible to depression, suicidal tendencies, aggression, cognitive impairment, substance abuse, poverty, anxiety and medical complications (Baldessarini, 2001; Mullen, 2006).

2.1.1. Types of Psychotic Disorders

Psychosis has been quite difficult to classify because there is no clear-cut classification of the different types of psychotic disorders. Diagnostic and Statistical Manual of Mental Disorders (DSM–III-R), a Copenhagen High-Risk Project on Psychotic Disorders gave the following classification (APA, 1987).

•CLASS I: Functional Psychosis

Functional psychosis consists of schizophrenic psychotic disorders and is the most severe form of the disease. It is characterized by delusions, thought disorders and hallucinations. Other types are schizophreniform psychosis, schizoaffective disorders and atypical psychosis (Baldessarini, 2001).

•CLASS II: Cluster 'A' Psychotic Personality Disorders

It has basic symptoms commonly referred to as the "four As" (loose associations, flat affect, ambivalence and autism). It includes paranoid personality and schizoid personality (Ollin, 1996; Allan and Robert, 2005). .Paranoid personality usually refers to as paranoia or delusional psychosis is characterized by fixed suspicions, persecutory delusions, dominant ideas or grandiose trends, which are false ideas. Pure paranoia is different from typical schizophrenia by formally correct conduct, coherence of the train of thoughts and adequate emotional reactions (delusional disorders in DSM- IV). There is no mental defect or dementia other than the delusional systems-hallucinations or emotional disturbances (Ollin, 1996). Schizoid personality is characterized by a tantrums and overly pious, shy, fearful, solitary, conscientious and idealistic- traits that may have marked these individuals as odd (Allan and Robert, 2005).

•CLASS III: Major Depression (Mood Disorders)

This could be unipolar hyperactive or depressed mood and bipolar depression. So also Peuperal (postpartum) psychosis and substance abuse psychosis (APA, 1987; Allan and Robert, 2005).

2.1.2. Historical perspectives of Schizophrenia

Emil Kraeplin, a Munich psychiatrist, was the one who first coined the word 'dementia-praecox' out of the array of manic–depressive psychosis in 1896. The word refers to deterioration of mental function at an early age, from a previous level of normalcy. He emphasized that the disease onset occurs in adolescence or early adult life and has a chronic course. He also established that 'catatonia' and 'hebephrenia' are both components of the disease (Kraeplin and Robertson, 1919). However, schizophrenia was later used to replace the word 'dementia praecox' by Eugen Bleuler. Bleuler, who also introduced the word 'autism' to indicate 'thoughts divorced from reality'as a part of thought disorder. Schizophrenia was characterized by the four As (loose associations, flat affect, ambivalence and autism) (Bleuler, 1955). Adolf Meyer and Sigmund Freud introduced the 'psychobiologic approach' to psychiatry and sought the origins of

schizophrenia as well as other psychiatric syndromes. They emphasized that habitual reactions to life events play a crucial role in the pathology of the disease (Allan and Robert, 2005). However, Langfeldt proposed the term schizophreniform psychosis to combine both the features of dementia praecox and schizophrenia (Allan and Robert, 2005).

2.1.3. Clinical Symptoms of Schizophrenia

There are three main mental and behavioural features of schizophrenia, which include the following:

•**Negative symptoms:** These include diminished motor activity or spontaneous movement and thought disorders e.g. fragmentation of ideas, loosening of associations, tangentiality and inappropriate emotional expression. Other negative symptoms include abulia or avolition (lack of motivation or drive), alogia (poverty of speech), anhedonia (lack of pleasure) and social withdrawal (Rollins *et al*., 2010). These symptoms tend to affect the quality of life of the patients more than the positive symptoms (Velligan , 2008).

•**Positive symptoms:** These consist majorly hallucinations and delusions (Liddle and Barnes, 1990). There is what is called circumstantiality (talks generally around an idea but never around the main issue of discussion), tangentiality (when thoughts goes off target and never return to the original thinking) and derailment (involves flunctuation of thoughts from one idea to another without any points connecting them). The patients have the difficulty in communicating their ideas clearly. In analysis of a problem, there is the tendency to be 'over inclusive' rather than 'under inclusive' (as in dementia). Over time, there is a general deterioration in functioning, social withdrawal and at times bizarre actions, idleness, self-absorption and aimlessness (Baldessarini, 2001; Mullen, 2006). In more severely affected schizophrenic patients, thinking is even more disintegrated, as the individuals appear to be totally preoccupied with their inner psychic life (thus the early use of the term autism) and only meaningless phrases or neologisms are present or mere 'word salad'. The patients cannot concentrate on any meaningful task because of sudden 'blocking 'and 'insertion' of extraneous ideas (Baldessarini, 2001; Mullen, 2006; Combs and Muester, 2007). There may be unusual ideas and experiences like his body belongs to another person termed 'depersonalization'. 'Thought insertion' i.e. as if an idea has

been implanted in his mind and 'thought withdrawal', feeling of ideas being extracted from his mind (Davis, 2001; Allan and Robert, 2005). Hallucinations are more peculiar to patients with schizophrenia; feelings of imaginary images or words or voices being heard only by them, recognizable and at times unrecognizable i.e. 'auditory hallucination' or seeing images by them alone 'visual hallucinations' (Allan and Robert, 2005).

•**Cognitive symptoms:** They are subtle symptoms and are characterized by poor ability to absorb and interpret information and decision making, often termed 'executive functioning'. It could also be inability to sustain attention and problems with being able to keep recently learned information in mind and use it the right away (Allan and Robert, 2005).

2.1.4. SUBTYPES OF SCHIZOPHRENIA

Schizophrenia has been grouped into the following subtypes although there are still some cases that do not conform entirely to these conventional subtypes (APA, 2000; Grohol and John, 2013).

•PARANOID SCHIZOPHRENIA

This is one of the most frequent types of schizophrenia with onset at about the age 40 and is a life long illness. However, sufferers can leave a fairly high quality of life with good treatment (Mayo, 2013). Presence of one or more delusions and auditory hallucination are the main features. The hallucination is often persecutory in nature with delusional jealousy as well. Majority has thinking disorders that may lead to mistrust and suspiciousness (Allan and Robert, 2005). The earlier the diagnosis and time of initiation of treatment, the better is the outcome.

•CATATONIC SCHIZOPHRENIA

This is characterized by extreme diminution in psychomotor activity and there is a stuporous catatonia or excited catatonia. Excited catatonia is a heightened aggression as part of its features in Africans but more of suicidal tendencies in the Western counterparts (Baldessarini, 2001). Excited catatonic patient talks or shout almost continuously. His verbal alteration are often incoherent and behavior is dependent more on inner impulses that from the environment (Davies

et al., 1991). However, stuporous catatonia is characterized mainly by complete stupor, slackening interest, apathy, preoccupation, loss of interest in food and tendency to maintain one position (Allan and Robert, 2005). Their lips often appeared pursed and if a limb is lifted up, it could assume that position for quite a long time (flexibilitas cerea). They may assume robot-like manner i.e. automatic obedience without hesitation. Negativism refers to patient's failure to cooperate without any clear reason (Davis *et al*., 1991).

•DISORGANIZED (HEBEPHRENIA) SCHIZOPHRENIA

It is either called Hebephrenia or disorganized schizophrenia. It occurs in earlier age compared to other types. 'Hebe' means 'Youth' in Greek and it occurs around the period of puberty (Maric, 2000). Hallucination is mainly auditory without discrimination of external stimuli and inner mental states. It is characterized by a mental state in which the patient is no longer aware of his own thoughts and purposes. Motor symptoms include stereotyped mannerism, grimacing, oddities of behaviour are also very prominent. Stereotyped behaviours involve purposeless, compulsive or strange behaviours or gesture performed over and over, repetitively (Szechtman, 1986). Tantrums in schizoid personality early in life are present (Allan and Robert, 2005). Volition ability is impaired (Baldessarini, 2001). Delusion entails illogical or highly idiosyncratic communications and irrational behaviours.

•SIMPLE SCHIZOPHRENIA

In this form, patients exhibit thought disorders, bland affect, withdrawal, and reduction in speech and movement, all of which impair work performance. Poverty of psychomotor activity is a dominant feature; however, hallucinations and delusions are absent (Solovay *et al*., 1987). Terms like schizoid, schizotypal, latent, or borderline schizophrenia have been applied to this type of schizophrenia (APA, 2000; Allan and Robert, 2005). Sufferers often like to be lonely 'loners'. They may drift from one job to the other and are always shy, withdrawn and relatively indifferent to their surroundings.

2.1.5. Epidemiological Profile of Schizophrenia

The distribution of a disorder in a given population is measured in terms of 'incidence' and 'prevalence'. Incidence is the proportion of new cases per unit of time (usually one year) while prevalence refers to the proportion of existing cases (both old and new). There are three types of prevalence rate. Point prevalence is a measure of the number of cases over a defined period of time (six months or one year); and lifetime prevalence – a measure of the number of individuals who have been affected by a disorder at any time during their lives (Jablensky *et al*., 2000).

Schizophrenia cases across the globe have been estimated at 29 million of whom, 20 million live in developing or underdeveloped countries. Point prevalence in adults range between 1 and 17 per 1000 population; while prevalence is between 1 and 18 per 1000. Earlier studies of prevalence suggest that at any given time, about 0.85 percent of world population is suffering from schizophrenia, expectancy rate is as high as one in hundred(Jablensky , 2000). Prevalence varies across the globe. Highest rate is in Africa (Nigeria especially) compared to Europe or America (Ewhrudjakpor, 2009). With only few exceptions, the economic burden present with psychotic disorders are remarkably similar among all cultures. However, it is perceived to be more common among Africans (Ewhrudjakpor, 2009).

Psychosis commonly has its onset in late adolescence or early adulthood but rarely occurs before adolescence or after the age of 40 years. Although the prevalence of psychosis has been shown to be equal in both males and females, the onset of illness tends to be earlier in males. Males most frequently have their first episode during their early twenties, whereas with females, it is usually during their late twenties to early thirties (APA, 2000; Jones and Buckley, 2003). For unknown reasons, the incidence is higher in social classes showing high mobility and disorganization. It has been suggested that this is as a result of a "downward drift'' due to deteriorating functions caused by the disease or socioeconomic factors (Allan and Robert, 2005).

Reports have shown that schizophrenic patients occupy about half the beds in mental hospitals- more hospital beds than are located to any other single disease and they constitute 20 to 30 percent of all new admissions to psychiatric hospitals (100,000 to 200,000 new cases per year in the United States) (Allan and Robert, 2005). The current prevalence of 29.3% is observed in a recent study compared to previous figures of 22.3% that was previously recorded in South- East , Nigeria (Okpataku *et al.*, 2014).

2.1.6. Risk factors associated with psychosis

Over the past few decades, the number of psychotic patients that has been institutionalized has significantly increased. Multiple factors interacting in a complex manner have been identified as the major causes for such increase (Olin and Mednik, 1996). This includes family psychiatric history, perinatal and obstetric complications (puerperal or postpartum psychosis), neurobehavioural deficits, institutionalization, and poor family functions (such as poverty and unemployment, family instability and early parental separation), substance misuse (drugs), and traumatic stress or life events (Olin and Mednik, 1996; Kabir *et al.*, 2004; Ewhrudjakpor, 2009). Several lines of evidence have shown that increased rick of psychosis is associated with increased emotional reactivity to the small stresses of daily life (Myin-Germeys *et al*., 2001; Myin-Germeys and van Os, 2007). Stress has also been implicated in the increased intensity of subtle psychosis-like symptoms in the realm of daily life (Myin-Germeys and van Os, 2007). These findings suggest that the association between stress and psychosis may be a consequence of an underlying vulnerability, characterized by increased emotional and psychotic reactions to stress. Interestingly, increased stress reactivity was found to be unrelated (even inversely related) to cognitive impairment, an intermediary phenotype associated with genetic risk for schizophrenia, thus suggesting the existence of different stress-and non-stress-related independent pathways to psychosis (Myin-Germeys *et al*., 2001; Morrens *et al*., 2007). The stress reactivity pathway, which has also been termed the "affective pathway to psychosis," has been hypothesized to preferentially underlie the positive symptoms of psychosis (Myin-Germeys and van Os, 2007). The impact of stress on the genesis of psychosis has been explained based on the concept of behavioural sensitization (Collip *et al*., 2008). Sensitization refers to the process whereby repeated exposure to a certain event increases the behavioural and biological response to a later exposure to a similar event, even if the later exposure is not severe (Collip *et al*., 2008). Increased emotional and psychotic reactions to stress may be the result of such a process of behavioral sensitization, occurring when previous exposures to severe or enduring stressors result in increased responses to the small stresses of daily life. Indeed, previous exposure to childhood trauma (Glaser, *et al*., 2006) or life events (Myin-Germeys *et al*., 2003) has been suggested to increase the sensitivity to small stresses in daily life; the cumulative impact of which may lead to the development of psychosis (Myin-Germeys and van Os, 2007). Furthermore, studies have shown that stress task cause a significant release of dopamine in the

brain . In addition, individuals who had experienced low maternal care also had increased striatal dopamine release in response to a social stress task compared with individuals with high maternal care (Pruessner *et al*., 2004).

2.1.7. Pathogenesis of Psychosis

Although the etiology of psychotic disorder still enjoys the luxury of universal discord, research has demonstrated various abnormalities in brain structures and functions (Harrison, 1999). However, these changes are inconsistent among all individuals with a diagnosis of psychosis, and much is yet to be learned about its pathogenesis. The cause of psychosis is likely multifactorial; that is multiple pathophysiological abnormalities may play role in producing the similar but varying clinical phenotype of psychotic disorders (Crismon and Buckley, 2005). One widely held contemporary hypothesis summarized in Figure 1 is that this disease reflects an underlying developmental disorder, determined either genetically or due to environmental insults, leading to abnormalities of synaptic connectivity, prominently affecting the hippocampus and prefrontal cortex (Allan and Robert, 2005; Lieberman *et al*., 2008). Additionally, decreased mitochondrial function, impaired glucose metabolism, reduced neurotrophic factors expression, altered neuroactive steroids and failure in intracellular signaling cascades have been proposed as potential triggers of psychosis (Steen *et al*., 2006; Beaulieu *et al*., 2007).

•Genetic Factor in the Pathogenesis of Psychosis

It has been shown that genetic factors may account for upward of 80 percent of the risk of developing schizophrenia (Allan and Robert, 2005). While the important of genetic linkage in the etiology of schizophrenia is undeniably possible, a Mendelian pattern of inheritance has not been determined. Within the last several years, polymorphisms in several genes have been implicated as risk factors of schizophrenia. Such genes include those expressing neuregulin, dysbindin, catechol-0-methyltransferase(COMT) and proline dehydrogenase (Harrison and Owen, 2003). The findings that allelic variants associated with specific neurotransmitter systems (in the genes that code for serotonin receptors and COMT) are overrepresented in schizophrenia tend to support genetic basis for the disease (Allan and Robert, 2005).

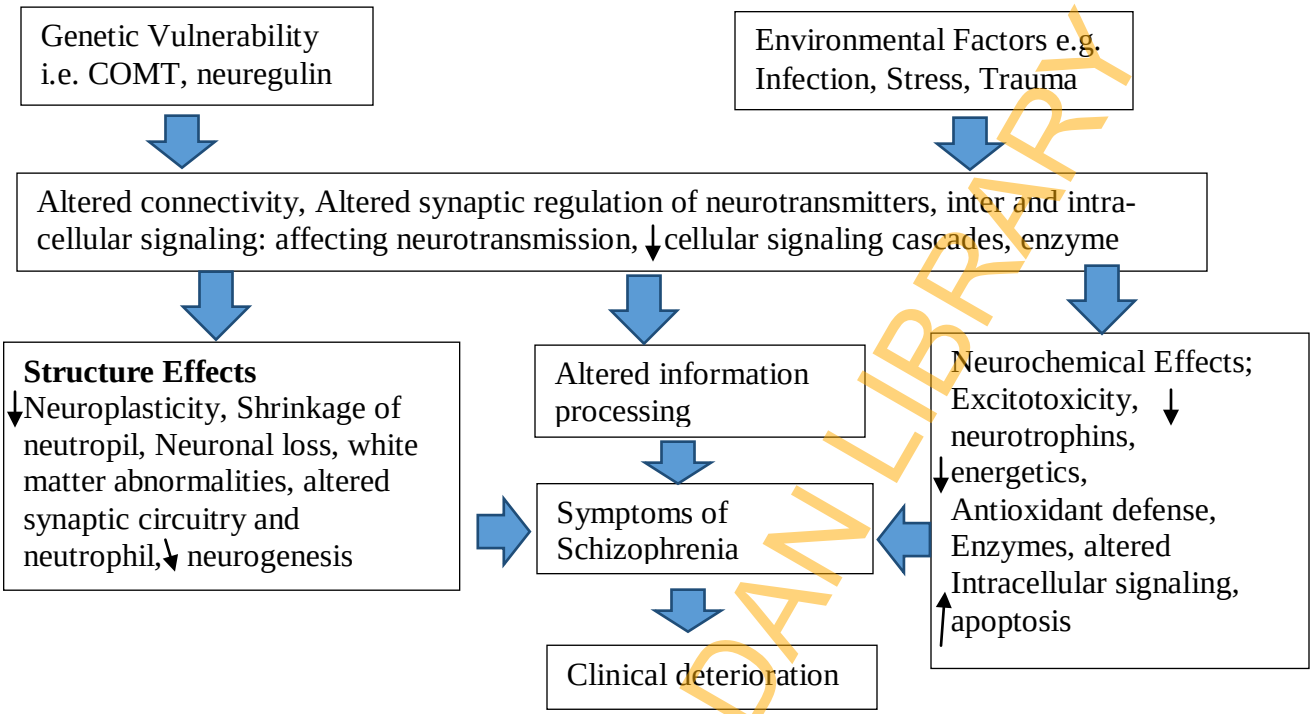

Fig. 1: Contemporary overview of the possible mechanisms of neurodegeneration occurring in schizophrenia (Lierberman *et al*., 1999).

•Neurodevelopmental theory of psychosis

Neurodevelopmental model have been proposed as one possible explanation for the etiology of psychosis (Lewis and Levitt. 2002). This model proposed that schizophrenic psychosis has its origins in some as yet unknown in utero disturbance, possibly occurring during the second trimester of pregnancy. Evidence for this has been provided by the abnormal neuronal migration demonstrated in most studies of schizophrenic brains. This ''schizophrenic lesion'' may result in abnormalities in cell shape, position, symmetry, and connectivity, and functionally to the development of abnormal brain circuits (Harrison, 1999; Lewis and Levitt, 2002). Other studies have shown a close relationship between obstetric complications, neonatal hypoxia as well as substance abuse and schizophrenia (Jones and Buckley, 2003). The resulting secondary "synaptic disorganization" associated with such insults is thought not to produce 'overt' clinical manifestations of psychosis until adolescence or early childhood, which also correspond to the period of neuronal maturation (Jones and Buckley, 2003; Harrison, 1999).

2.1.8. Implications of Neurotransmitter Abnormalities in the Pathology of Psychosis

The evolution of biological concepts for the pathogenesis of chronic mental illnesses was stimulated by the observations that certain psychoactive compounds modulates mood or behavior and mimic most of the symptoms of psychosis (Baldessarini, 2001). Extensive studies have shown the implicative role of these compounds in producing behavioural changes, through the biogenic amine pathways (Baldessarini, 2001). The discovery of substances that could block the actions of these psychoactive agents and thereby alleviate the symptoms of psychosis further supports the biological hypothesis of mental illness (Baldessarini and Tarazi, 2001).

•Dopamine Hypothesis of Schizophrenia

The major biogenic amine implicated so far has been dopamine, which comprises of five major receptor subtypes (D_1, D_2, D_3, D_4, D_5), although the others have also shown to be involved in the regulation of mood or behaviour (Davis *et al.*, 1991; Baldessarini and Tarazi, 2001; Owens, *et al.*, 2005). It is usually synthesized from the dopaminergic neuron as shown in Figure 2.2. Hyperactivity of dopamine has been the leading biochemical hypothesis and has been based on the response of psychotic symptoms to phenothiazine medications, thereby implicating the

dopaminergic system of the temporal lobe (Davis *et al*., 1999; Baldessarini and Tarazi, 2001; Owens, *et al*., 2005). Evidence for this is circumstantial and is supported by observations that antipsychotic drugs (such as the neuroleptics) reduced the electrical activity of mesolimbic dopaminergic neuron in the brain of patients with psychosis (Kapur 2003). A sensitization process involving dopaminergic dysregulation of key brain areas has been proposed as the common pathway leading to psychosis (Howes *et al*., 2004) and, indeed, as a potential model for schizophrenia including its cognitive and negative symptoms (Featherstone *et al*., 2007). Positron emission tomography (PET) studies have shown that drug-naïve schizophrenic patients display greater striatal dopamine release than controls when given amphetamine. This correlate with the severity of experimentally-induced psychotic symptoms as well as with the response to subsequent antipsychotic treatment (Laruelle *et al*., 1996; Abi-Dargham, *et al*., 2004). Radioligand-binding assays have also been used to determine more precisely the mode of actions of neuroleptic drugs, the clinical efficacy of which has been shown be associated with dopamine D_2 receptors (Davis *et al*., 1991; Baldessarini and Tarazi, 2001). Furthermore, evidences from histochemical studies have also lend support to the dominergic hypothesis as there have been several demostrations showing increased concentrations of dopamine or its metabolite; homovanillic acid, in schizophrenic brains obtained at autopsy. The ability of neuroleptic drugs to inhibit stereotyped behavior induced by dopaminergic agents such as apomorphine, or amphetamine further supports the dopaminergic hypothesis (Shopsin *et al*., 1979; Baldessarini and Tarazi, 2001). It is known that dopamine receptors are organized in two systems; the limbic and cortical. And it has led to the notion that an excess of dopaminergic activity in the mesolimbic system gives rise to the positive symptoms of schizophrenia whereas a diminished activity in the mesocortical system accounts for the negative symptoms (Allan and Robert, 2005).

2.1.9. Other neurotransmitters implicated in the Pathogenesis of Psychosis

• Gamma-Amino-Butyric Acid (GABA) and schizophrenia

GABAergic synapses have been found to be the key inhibitory synapses within the brain. Decreased GABAergic neurotransmission has been implicated in the pathophysiology of schizophrenia (Lewis *et al*., 2004; Guidotti *et al*., 2005). It has been proposed that deficits in

GABAergic neurotransmission may result in an imbalance between excitatory and inhibitory neurotransmission, favouring excitation and possible excitotoxicity (Guidotti *et. al*., 2005). Most studies have reported low GABA levels in at least some brain regions in patients with schizophrenia, although there is no clear consensus on the specific brain loci affected with the exception of the amygdale (Guidotti *et al.*, 2005). At the presynaptic level, down-regulation of mRNA or protein for Glutamate decarboxylase (GAD) has been observed in postmortem brain tissues of patients with schizophrenia. Olney *et al.*, (1999) suggested that a developmental deficit of inhibitory GABA interneurons may set the stage for ongoing neurodegeneration through the uncontrolled activation of glutamatergic neurons. In addition, GABAergic interneurons has been shown to play an important role in regulating pyramidal neuron firing rate (McBain and Fisahn, 2001). Reduced GABAergic function would alter the synchronous firing patterns of cortical neurons, which may underlie information-processing deficits known to be present in patients with schizophrenia (Hajos, 2006). GABAergic dysfunction in schizophrenia has been characterized as a reduction in the availability of GABA and related proteins presynaptically and compensatory upregulation of GABA receptors postsynaptically (Benes *et al.*, 1996).

•Glutamate dysfunction in schizophrenia

The involvement of glutamate in schizophrenia was derived from the psychosis syndrome produced by chronic ingestion of phencyclidine (PCP), an N-Methyl-D-Aspartate (NMDA) antagonist. NMDA receptors play a crucial role in axonal signaling during neurodevelopment (Rakic *et al*., 1994). Despite playing important role in diverse physiological functions, NMDA mediated glutaminergic neurotransmission is related to various pathologies, including neurodegenerative diseases, ischemia, epilepsy and schizophrenia (Meldrum, 1994; 1995; Ozawa *et al*., 1998). The implication of glutamate involvement in psychosis has received several favorable arguments in support of glutamate hypoactivity (Meador-Woodruff and Healy, 2000). However, dopaminergic and glutaminergic systems have been shown to converge on certain cortical neurons and that glutaminergic function is modulated in several regions of the brain by dopamine (Allan and Robert, 2005). Cognition involved neuronal plasticity is known to be mediated via NMDA receptors and schizophrenic patients showed marked deficits in cognitive functions (Daw *et al*., 1993). Zirpursky *et al.* (1992) has also linked a reduction in the grey

matter shown in the brains of schizophrenics to NMDA-mediated neurotoxicity. Additionally, ketamine (an NMDA receptor antagonist) has been shown to produce an increase sensibility to audio hallucinations in schizophrenics, perhaps by accentuating glutamatergic deficits in neurons linked to audition (Krystal *et al*., 2003). Therefore, a variety of symptoms found in schizophrenia could be partially explained through an alteration of NMDA-mediated glutamatergic neurotransmission (Meador-Woodruff and Healy, 2000).

•Serotonin (5HT) and noradrenaline hypothesis of schizophrenia

More recently, a hypothesis based on changes in the serotonergic system has been proposed. As with the dopaminergic model, attention was drawn to mechanisms relating to serotonin when a new class of antipsychotics (clozapine, risperidone), which have effects on the latter system, were found to ameliorate psychotic symptoms (Allan and Robert, 2005). Evidence from several studies showed alterations in serotonin receptors in the brains of schizophrenic patients (Allan and Robert, 2005). Further studies revealed that an allelic variation in the gene on chromosomes '13' encoding for a serotonin receptor (5-HT_{2A}) confers susceptibility to schizophrenia (Williams *et al*., (1996). A similar finding has been reported in a Japanese population. However, the variation in this gene is not sufficient enough to explain the presence of the disease in any one individual, if for no other reason than that many patients who are homozygous for the suspected allele do not develop schizophrenia (Allan and Robert, 2005). Also, Noradrenalin (NA), has been implicated in the pathogenesis of schizophrenia (Friedman *et al*., 1999; Yamamoto and Hornykiewicz, 2004).Increased noradrenaline levels has been found in Cerebrospinal Fluid , autopsy brain and plasma in psychosis (Jaap *et al*., 2012).

2.2.0. ANIMAL MODELS OF PSYCHOSIS

Several models have been established for screening of compounds with antipsychotic property in experimental animals and they include the following:

•Antagonism of stereotypy induced by pharmacological agents in rodents

This model is commonly used in the experimental study of psychosis in animals and it involves the injection of certain pharmacological agents (e.g., amphetamine, bromocriptine, apomorphine, ketamine) to induce stereotype behavior, a prominent symptom seen in patients with psychosis (Szechtman, 1986; Davis *et al*., 1991). Stereotypy involves the performance of series of purposeless, compulsive or strange behaviors or gestures over and over again, such as asking the same question or making the same comments repetitively (Szechtman, 1986; Davis *et al*., 1991). However, in animals, it may be manifested as persistent head movements, intermittent sniffing, persistent chewing and intense linking (Szechtman, 1986). The antagonism of amphetamine induced stereotyped behavior is a well-known animal model employed in the screening for compounds with anti-psychotic properties (Waddington and Gamble, 1980; Bourin *et al*., 1986; Siqueira *et al*., 1986). The ability of antipsychotic drugs to attenuate stereotyping has been ascribed to the antagonism of dopaminergic system (Shopsin *et al*., 1979; Bourin *et al*., 1986; Davis *et al*., 1991).

•Inhibition of hyperactivity induced by amphetamine in rodents

Studies have shown that inhibition of amphetamine-induced hyperactivity is of benefit in antipsychotic drugs (Siqueira *et al*., 1998). This test is commonly performed using open field apparatus and the duration of ambulation as well as the distance travelled are recorded. Antipsychotics are known to reduce hyperactivity induced by amphetamine in rodents (Bourin *et al*., 1986; Siqueira *et al*., 1998).

•Protection against amphetamine-induced lethality in animals

The increased lethality induced by amphetamine in grouped mice, is considered to be a specific model for psychosis since only antipsychotic agents but not minor tranquilizers (benzodiazepines and barbiturates) can protect animals against death. This protective effect is known to be closely related to blockade of D_2 dopamine receptors (Burn and Hobbs, 1958; Bourin *et al.*, 1986).

2.2.1. Agents used for the induction of psychosis in experimental animals

Agents commonly used to induce psychosis in experimental animals include apomorphine, amphetamine, ketamine and bromocriptine (Rang *et al.*, 2003). Hallucinogens like Lysergic acid diethyl amine (LSD), phencyclidine and psilocybin also produce psychosis.

2.2.2 Mechanism of Action of Bromocriptine

Bromocriptine is an ergot-derived dopamine agonist. Its current uses include the treatment of Parkinson's disease, postpartum ablaction, prolactionmas, acromegaly, amenorrhea and galactorrhea secondary to neuroleptic use. It is often reported to produce psychiatric side effects such as confusion, hallucinations, and delusions (Alan-Boyd, 1995). Bromocriptine has the longest history of use for the treatment of hyperprolactinemia and is well established as a safe and effective therapy. Psychiatrists have been reluctant to use drugs that have dopamine agonist properties, particularly bromocriptine, however, for fear of exacerbating the patient's psychiatric symptoms. It has been reported in a small number of studies that when administered for the treatment of antipsychotic-drug-induced hyperprolactinemia, bromocriptine precipitates a psychotic state (Moo-Soo Lee *et al*., 2010).

2.2.3. Bromocriptine-induced Psychosis

Bromocriptine is often reported to produce psychiatric side effects such as confusion, hallucinations, and delusions. This supports a strong anecdotal relationship between bromocriptine use and psychosis (Alan-Boyd, 1995). Bromocriptine was shown to interact with dopaminergic system evidenced by the correlations between substantia nigra, dopamine cell activity and striatal extracellular dopamine level in rats (Holly *et al*., 1998; Lierberman *et al*., 1985). Acute administration of bromocriptine decreased spontaneous motor activity while subacute doses increased spontaneous motor activity (Al-Gdamsi and Aburawi, 2013). The activity of bromocriptine has been investigated in tests for the stimulation of central dopaminergic mechanisms. The results obtained were compared with those of apomorphine, amphetamine and L-DOPA. Bromocriptine (2.5 to 10 mg/kg, i.p) induced stereotyped sniffing and licking in rats. The stereotypy was shown to be more intense than that induced by L-DOPA but less intense than that of apomorphine and amphetamine (Johnson *et al*., 1976). In rats lesioned unilaterally in the substantia nigra by local injection of 6-hydroxydopamine, bromocriptine, like apomorphine and L-DOPA, induced turning contralateral to the side of the lesion. Reserpine-induced catalepsy in mice was also antagonized by bromocriptine in a similar manner to apomorphine and L-DOPA. Spontaneous locomotor activity in mice was stimulated by bromocriptine in a dose-dependent manner from 2.5 to 10 mg/kg after an initial suppression of activity. In all experiments, bromocriptine was characterized by a prolonged duration of activity after a delay in the onset of effect. The stereotyped behaviour induced by bromocriptine was inhibited by prior administration of pimozide, reserpine or a-methyl-p-tyrosine (Johnson *et al*., 1976). Bromocriptine-induced turning behaviour was abolished by pretreatment with pimozide, and reduced after a-methyl-p-tyrosine pretreatment. The results obtained support the conclusion that bromocriptine acts by stimulating dopamine receptors in the central nervous system and that intact catecholamine synthesis and granular amine storage mechanisms are necessary for its psychotic effects. Other effects of bromocriptine include inhibition of prolactin secretion and glutamate release (Missale *et al*., 1998). Bromocriptine also demonstrated antioxidant and neuroprotective properties in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated animals (Krishnamurthy and Archer, 2003).

2.2.4. Therapeutic Approach to the Treatment of Psychosis

Goals of acute and maintenance treatment are the reduction of disorganized, agitated or hostile behaviours, reduction of social withdrawal and decreasing the impact of hallucinations (Meltzer, 1996). Different categories of pharmacological treatments and psychosocial treatment exist and they target specific set of symptoms of schizophrenia (Kane and Corell, 2010). Treatment plan is fashioned for each patient (Bloch *et al*., 2012).

2.2.5. Classification of Antipsychotic Drugs

Generally, antipsychotic drugs are classified into two major groups: typical and atypical antipsychotics (Meltzer, 1996).

• Typical (First-Generation) Antipsychotic Drugs

Typical antipsychotics are categorized into high and low potency, where high potency (*e.g.* haloperidol) allows for greater schizophrenic symptom relief but has many neurological side effects, including various form of dyskinesia. Low potency (*e.g.* chlorpromazine) is less adversely effective; however they still target or possess high affinity for histaminic (H_1), muscarinic (M_1) and alpha-1 (α_1) receptors that cause many of their undesirable effects (sedation, anticholinergic properties and orthostasis (Leucht *et al*., 2009). They are generally known to produce adverse effects similar to symptoms of Parkinson's disease (tardive dyskinesia and body rigidity, Leucht *et al*., 2009). This group of drugs includes phenothiazines (chlorpromazine, promazine, trifluopromazine; thioridazine, mesoridazone, fluphenazine, trifluopenthixol, perphenazine and prochlorperazine); butyrophenones (haloperidol, trifluperidol, penflurifol, droperidol) and thioxanthines (chlorprothiexe, thiothixene, flupenthixol).

- **Atypical (Second Generation) Antipsychotics**

They are known as atypical antipsychotics for their little propensity to cause extrapyramidal adverse effects. They include bensizisozazole(risperidone); thionobenzodiazepam (olanzapine); dibenzodiazephines (clozapine); dibenzozazepine (lozapine); diphenylbutyipiperidine

(pimoxide); dibenzothiazepine (quetiapine); dihydroindolone (ziprasidene and sertindone) and dihydrocarbostyril (aripiprazole) (Baldessanni and Tarazi, 2001; Leucht *et al*., 2009). However, their high level of efficacy is compromised by the incidence of increase in metabolic effects (*e.g.* weight gain, precipitation of diabetes etc.) as well as higher cost of production (Kane and Corell, 2010).

2.2.6. Mechanism of Action of Antipsychotics

Emerging data indicate that stimulation of glutamate or muscarinic receptors may confer antipsychotic properties but no clinically available effective antipsychotic is devoid of D_2 antagonistic activity. This is because antipsychotic agents mediate their action by blocking mainly D_2 dopamine receptor or by blocking preferentially D_2 dopamine receptor (Goodman and Gilman, 2012). Although, some may also block other monoamine receptors such as serotonin, histamine and α-adrenoceptors. The potency of antipsychotic agents has shown good correlation with their capacity to bind to D_2 receptor. The degree of this blockade is related to other effects seen due to interaction with other receptors like 5-HT. ACh determines their ability to ameliorate or alleviate the positive, negative, and cognitive symptoms, which varies considerably between drugs (Leucht *et al*., 2009). Phenothiazines and thioxanthenes also block D_1, D_3 and D_4 receptors but still there is no correlation with antipsychotic potency. Some may show selective actions (*e.g.* the typical have preference for D_2 receptors over those of D_1; while some of the newer agents like sulpiride exhibit high infinity for D_2 receptor). Clonazepine display a relative non-selectivity action between D_1 and D_2 receptors but very high affinity for D_2 receptors. Still, they are some that show equal-affinity for both D_2 as well as serotonin ($5\text{-}HT_2$) receptors (risperidone, sertidone, and ziprasidone). However, aripiprazole remain the only current drugs with D_2 antagonistic and partial D_2 agonistics effect (Baldessarini and Tarazi, 2001).

The reduction in dopaminergic neurotransmission is presently achieved through one of two mechanisms: D_2 antagonism or partial D_2 agonism (Goodman and Gilman, 2012). Blockage of dopaminergic projections to the temporal and prefrontal areas constituting the 'limbic system' and in mesocortical areas is probably responsible for the antipsychotic action (Leucht *et al*., 2009). Aripiprazole's capacity to stimulate D_2 receptors in brain areas where synaptic dopamine levels are limited *e.g.* Prefrontal Cortex (PFC) neurons or decrease dopaminergic activity when

dopamine concentrations are high (e.g. mesolimbic cortex) is thought to be the basis for its clinical effects in schizophrenia (Goodman and Gilman, 2012). It has been reported that the blockage of dopamine receptors by chlorpromazine and related drugs occur in the neostriatum and in the limbic systems (Davis *et al*., 1991). This blockade of D_2 receptors has been shown to be responsible for the effectiveness of neuroleptic drugs in the treatment of psychosis. However, the reciprocal blockage of striatal D_2 dopamine receptors is responsible for the genesis of extrapyramidal side effects produced by chlorpromazine and related drugs (Beladessarini and Tarazi, 2001; Kane and Corell, 2010).

The low tendency of atypical antipsychotics to cause little or no extrapyramidal side effect is due to their low affinity for D_2 dopamine receptors (Davis *et al*., 1991 ; Beladessarini and Tarazi, 2001 ; Kane and Corell, 2010) and affinity for 5-HT_2, M_1 and α_1 blocking action, and some are relatively selective for D_4 receptors (Kane and Corell, 2010). Thus, antipsychotic property may depend on a specific profile of action of the drugs on several neurotransmitter receptors. Clozapine, by way of contrast showed a lower level of D_2 receptor occupation with lesser incidence of EPS (Beladessarini and Tarazi, 2001). However, the effectiveness of clozapine is grossly impaired due to the high incidence of agranulocytosis (Leucht *et al*., 2009).

2.2.7. Pharmacological Properties and Adverse Effects of Antipsychotics

In a psychotic patient, antipsychotic reduces irrational behaviour and agitation, and controls psychotic symptomatology. Disturbed thought and behaviour are gradually normalized, anxiety is relieved. Hyperactivity, hallucinations and delusions are suppressed (Goodman and Gilman, 2012). All phenothiazines, thioxanthenes and butyrophenones have the same antipsychotic efficacy, but potency differs in terms of equieffective doses. The aliphatic and piperidine side chain phenothiazines have low potency, produce more sedation and cause greater potentiation of hypnotics, opioids etc. The sedative effect is produced promptly, while antipsychotic effect is known to take weeks to develop. Moreover, tolerance develops to the sedative but not to the antipsychotic effects. Thus, the two appear to be independent actions (Kane and Corell, 2010).

Performance and intelligence are relatively unaffected, but sideways vigilance is impaired. A predominance of lower frequency waves occurs in EEG (Electroencephalogram) and arousal response is impaired. The disturbed sleep pattern in a psychotic patient is normalized (Beladessarini and Tarazi, 2001). Temperature control is knocked off at relatively higher doses and body temperature becomes dependent on surrounding. The medullary respiratory and other vital centers are not affected, except at very high doses (Beladessarini and Tarazi, 2001). Neuroleptics, except thioridazine, have potent antiemetic action exerted through the chemoreceptor trigger zone (CTZ). However, they are ineffective in motion sickness (Kane and Corell, 2010).

Extrapyramidal Symptoms (EPSs) is the most serious adverse effect in terms of frequency and reason for patients' non-compliance of antipsychotic drug therapy. Three categories of EPSs are usually manifested; dystonic reaction, pseudo-parkinsonism and akathisia. Dystonic reaction include oculogyric crisis (fixed upward gaze); torticollis (necktwist); opisthotonus (high aching of back) and trismus (inability to open jaws). Pseudo-parkinsonism include akinesia-rigidity and immobility, stiffness and slowness, stooped posture, shuffling gait, slow monotonous speech and tremo-regular rhythmic oscillations of extremities especially hand and fingers. Akathisia is manifested as the inability to sit still, constant pacing, continuous agitation of restless movement, rocking shifting of waist while standing; sifting of leg (Kane and Corell, 2010).

2.2.8. Oxidative Stress

Oxidative stress is the condition arising from imbalance between toxic reactive oxygen species (ROS) and antioxidant defense system (Halliwell *et al.*, 2006; Wood *et al.*, 2009). Oxidative stress occurs when cellular antioxidants defense mechanisms fail to counterbalance and control endogenous reactive oxygen species (ROS) and reactive nitrogen species (RNS) generated from normal oxidative metabolism or from pro-oxidant environmental exposure (Kohen and Nyska, 2002). A free radical is specie containing one or more unpaired electrons with the ability to exist independently (Halliwell, 2006). Those most often found are ROS and RNS. Oxygen, because of its bi-radical nature, readily accepts unpaired electrons to give rise to a series of partially reduced species collectively known as ROS [superoxide anion (O_2), hydrogen peroxide (H_2O_2),

hydroxyl ion (H)] and RNS [nitric oxide (NO), peroxynitrite (NOO)] until it is itself completely reduced to water (Singh *et al*., 2004; Wood *et al*., 2009).

These free radicals play integrated roles in cellular signaling, physiological, immunological responses and mitosis. However, being highly unstable molecules with unpaired electrons, they have differential oxidative strengths and hence potential to damage cellular proteins, lipids, carbohydrate and nucleic acids (Filomeni, 2006). Several aspects of defense exist to protect against these free radicals, including removal by non-enzymatic and enzymatic antioxidants (Davies, 1995; Wood *et al*., 2009). Despite the efficacy of this multi-faceted defense network, a degree of oxidative damage is inherent in aerobic life and is believed to underlie the pathophysiological mechanism of most psychiatric diseases (Wood *et al.*, 2009).

2.2.9. Oxidative Stress in Schizophrenia

The brain is known to be the most metabolically active tissue in the body and generates a high load of reactive oxygen moieties (Wood *et al.*, 2009). Brain weighs only 20% of the body weight but it consumes 20% of the body oxygen and 25% of body glucose at rest (Clark and Sokoloff, 2009). Reactive oxygen species (ROS) produced in any tissue is directly proportional to its oxygen consumption which further increases with intellectual process like thinking, planning, and reasoning (Ikonomiodou and Kaindi, 2001). Thus, the brain is persistently under oxidation/antioxidant process, which makes it prone to oxidative damage (Wood *et al.*, 2009; Bókkon and Antal, 2011).

The mechanism through which dopamine contributes to the formation of ROS (loss of redox balance) is via its spontaneous autoxidation (Cadet and Brannock, 1998). The dopamine molecule of the catechol group can easily be oxidized non-enzymatically to form a series of electrochemical type quinoide species. The initial step in the oxidation of the dopamine involves a reaction with molecular oxygen to form two molecules of the superoxide anion and dopamine-O-quinonen (Selva *et al*., 2011). The formation of the superoxide anions during the autoxidation of the dopamine leads to production of H_2O_2 by the dismutation of superoxide by SOD, which may react with transition metals ions, via the Haber Weiss/Fenton reactions, leading to the

formation of highly toxic hydroxyl radical (.OH) that causes lipid peroxidation (Wood *et al*., 2009).

Although a clear mechanism underlying the pathogenesis of schizophrenia remains unknown, oxidative stress as a consequence of aberrant redox controls has become an attractive hypothesis, at least in part of the pathophysiology of schizophrenia (Behrens *et al*., 2009; Zhang *et al*., 2010). This hypothesis has theoretical appeal, as the brain is considered particularly vulnerable to oxidative damage for several reasons. These include its comparatively high oxygen utilization and hence generation of free radicals, high metabolic activity, low levels of protective antioxidant enzymes, a high ratio of membrane surface area to cytoplasmic volume, neuronal anatomical network vulnerable to disruption, high proportion of readily oxidizable membrane polyunsaturated fatty acids (PUFAs), auto-oxidizable neurotransmitters (dopamine, adrenaline and noradrenaline) and presence of redox-catalytic metals (iron and copper) (Halliwell, 2006). Additionally, the brain is also susceptible to secondary and self-perpetuating damage from oxidative cellular injury or neurosis via the neurotoxic effects of released excitatory amines (mainly glutamate) and activated inflammatory cells (Halliwell, 2006). Metabolism of neurotransmitters generates large amounts of hydrogen peroxide (H_2O_2) and neuronal mitochondrial can generate superoxide radical (Mahadik and Mukherjee, 1996; Halliwell, 2006).

2.3.0. BIOLOGICAL PROPERTIES OF METHYL JASMONATE

Jasmonates are plant stress hormones that have been found to be involved in the defense of plants against external stress (Fingrut and Flescher, 2002; Rotem *et al*., 2005; Cohen and Flescher, 2009). They are important in the regulation of the physiological processes that are critical to the development and survival of plants. Jasmonates are cyclopentanone compounds and are synthesized in plants from linolenic acid through lipooxygenase pathway (Creelman *et al*., 1997). The role of jasmonates in stress is related to the formation of proteinase-inhibitor (PI) proteins in leaves of several plant families (Bowles, 1990). The PI proteins are array of defensive chemical substances that protect plants against pest, bacterial infections and other environmental stressors (Cohen *et. al*., 1993). The biosynthesis of PI proteins is trigger by the liberation of a

chemical substance known as systemin in response to exposure of plants to stress or when they suffer injury (Bowles,1990). Systemin is known to interact with plant plasma receptors, which results in the stimulation of lipase and the release of linolenic acid into the cytoplasm (Cohen *et al*., 1993; Rotem *et al*., 2005). Linolenic acid is inturn converted to jasmonates, which is postulated to interact with a receptor to activate PI gene expression, thereby providing resistantance-related responses against stress for wide variety of plants (Bowles, 1990). Jasmonates therefore, provide signals for the induction of proteinase inhibitor gene expression in the leaves of wounded plants, which acts as cellular messenger during plant defence responses (Fingrut *et al*., 2005).

Jasmonates were first discovered in the beautiful jasmine flower and were first isolated from Jasminium grandiflorum in 1962 (Damole *et al*., 1962). However, these plant stress hormones have been shown to be widely distributed throughout plant kingdom and certain microorganisms such as fungi and bacteria (Cohen *et al*., 1993). .Jasmonates are involved in regulation of plant growth and development. They also provide protection for plants against infections and mechanical injury. The levels of jasmonates have been shown to increase rapidly in response to external stress and when plants suffer injury or pathogenic invasions (Rotem *et al*., 2005).

Methyl jasmonate (Fig. 2) is the most well studied plant stress hormone belonging to the jasmonate family and several investigations have shown that it has potent anticancer property on a large spectrum of human cancer cells (Fingruts and Flescher, 2002; Rotem *et al*., 2005; Cohen and Flescher,2009). It has been found to significantly increased the survival of lymphoma-bearing mice and induced death in human leukaemia, prostate, breast and melanoma cell lines (Fingruts and Flescher, 2002) as well as in leukaemic cells from chronic lymphocytic leukaemia patients (Rotem *et al*., 2005). These findings support the importance of studying jasmonates as anticancer agents that can potentially eradicate tumors resistant to radiation and to currently available chemotherapy. The mechanism of action of jasmonates involves direct action on mitochondria, resulting in cell death (Rotem *et al*., 2005). Mitochondria are responsible for energy synthesis within cells, which have been discovered as a potential target for cancer therapy (Rotem *et al*., 2005). In contrast to mitochondria from most normal tissues, cancer cells mitochondria display association between the glycolytic enzyme hexokinase and the voltage dependent anion channel. This association is an important feature of the overactive bio-energetic

activity of tumor cells. Jasmonates cause detachment of hexokinase from its mitochondrial anchor in tumor cells, leading to disruption of energy generation in these cells and consequently to cancer cell death (Fingrut and Flescher, 2002; Rotem *et al*., 2005; Cohen and Flescher, 2009). It acts directly and selectively on human cancer cells while sparing normal cells would make it a more useful anticancer agent ever discovered (Fingrut and Flescher, 2002; Rotem *et al*., 2005). Combination of methyl jasmonate with conventional chemotherapeutic drugs was found to result in super-additive cytotoxic effects on several types of cancer cells (Cohen and Flescher, 2009). It was also found to cause cancer cells death in spite of drug-resistance conferred by either p53 mutation or P-glycoprotein over-expression (Cohen and Flescher, 2009).

Literature search also showed that methyl jasmonate also exhibits anti-parasitic property. It shows cytotoxic effect against two human parasites, *Schistosoma mansoni* and *Plasmodium falciparum* with *P. falciparum* being the more susceptible. Jasmonates did not cause any damage to control human erythrocytes at maximum concentration that was used in the studies (Gold *et al*., 2003). It was also found to be cytotoxic towards a metronidazole-resistant strain of *T. vaginalis* (ATCC 50143), suggesting that it may be effective for the treatment of nitroimidazole-refractory trichomoniasis. It caused fragmentation and condensation of DNA of T. vaginalis through perturbation of the bio-energetic homeostasis of the parasites (Ofer *et al*., 2008).

However, it was reported that MJ exhibits sedative effect and potentiates GABA-mediated inhibitory neurotransmission, which suggest its potential usefulness in neuropsychiatric disorders (Hossain *et al.*, 2004). Moreover, previous studies showed that intraperitoneal injection of MJ elicits antidepressant, anti-aggressive, adaptogenic/anti-stress and memory enhancing effects in experimental animals (Umukoro *et al*., 2011; Umukoro *et al*., 2012; Eduviere *et al* 2015; Umukoro *et al*., 2016) suggesting potential benefits in the alleviation of some of the symptoms of psychosis.

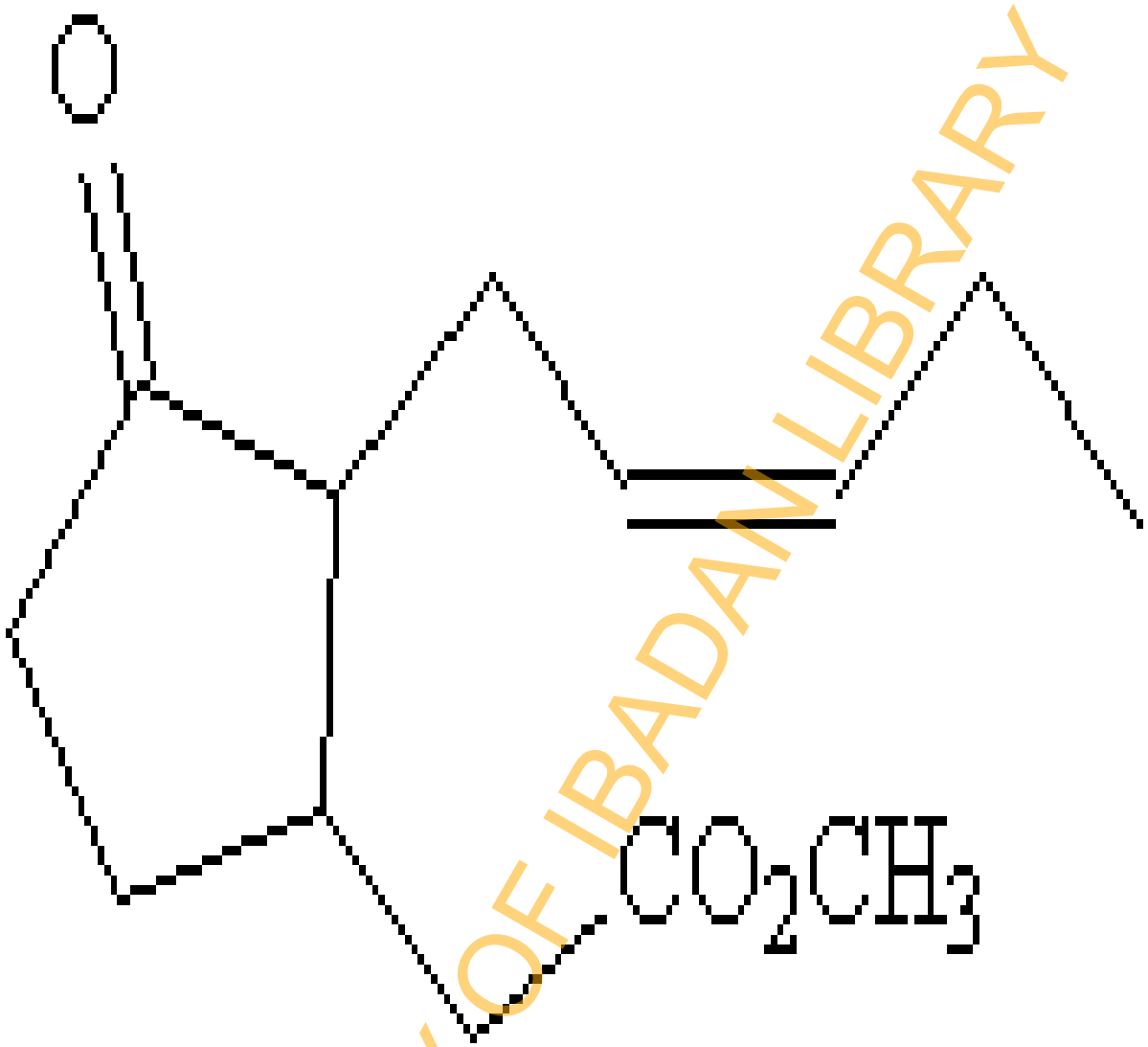

Figure 2: Chemical structure of Methyl jasmonate (Avanci *et al.*, 2010).

CHAPTER THREE

3.0. MATERIALS AND METHODS

3.1. Experimental Animals

Male Swiss mice weighing 23.5±1.5 g used in the study were obtained from the Central Animal House, University of Ibadan, Nigeria and were housed in plastic cages at room temperature with 12:12 h light–dark cycle. They were fed with balanced rodent pellet diet (Capsfeed, Ibadan, Nigeria) and water *ad libitum*. The animals were acclimatised for at least one week before commencement of experiments. The experimental procedures were performed in accordance with the NIH Guideline for the Care and Use of Laboratory Animals (National Academy of Sciences .USA. 2011).

3.2. Equipment and Apparatus

Centrifuge (ATKE), water bath (Equitron), spectrophotometer (Inesa, 752N), pH meter (EDT Instruments), weighing balance (Ohaus), test tubes, eppendorf tubes, tube racks, dissection kits and boards.

3.3. Drugs and Chemicals

Methyl jasmonate-MJ (Sigma, Darmstadt, Germany), bromocriptine (Meda, Solna, Sweden), ketamine (Rotex-medica, Trittau, Germany), 5,5′-dithio-bis(2-nitrobenzoicacid)-DTNB (Aldrich, Darmstadt, Germany), trichloroacetic acid-TCA (Burgoyne Burbidges & Co., Mumbai, India), thiobabituric acid-TBA (Guanghua Chemical Factory Co. Ltd., Liaoyang, China), Tris (hydroxymethyl)-amino-methane (Tris-buffer) (Hopkin & Williams Company, Surrrey, USA), Acetic acid (Sigma-Aldrich, Inc., St Louis, USA), $NaHCO_3$ (BDH Chemicals Ltd, Poole, England), Sodium Carbonate (Fisons, Loughborough Leics, England), $Na_2HPO_4.H_2O$ (BDH Chemical Ltd, Poole, England), $NaH_2PO_4.H_2O$ (BDH Chemical Ltd, Poole, England), K_2HPO_4 (BDH Chemical Ltd, Poole, England), $K_2Cr_2O_7$ (BDH Chemical Ltd, Poole, England), KCl

(BDH Chemical Ltd, Poole, England) and NaOH (J.T Baker Chemicals Co., Phillipsburg, N.J., USA) were used in the study.

3.4. Drug Preparation and Treatment

Methyl jasmonate (MJ) was prepared according to the procedure previously described by Umukoro *et. al.* (2012). Briefly, 0.5 mL of MJ was dissolved in 4.5 mL of ethanol (95 %) to obtain a stock solution. The stock solution obtained was further diluted with distilled water to attain the concentration used in the study. Also, the other drugs used in the study were dissolved in distilled water before use. The dose of 25, 50 and 100 mg/kg of MJ used in the study were selected based on the results obtained from preliminary investigations (Anthony *et al.*, 2015).

3.5. Behavioural Tests

3.5.1. Effect of MJ on Bromocriptine-induced stereotypy

Bromocriptine-induced stereotyped behavior was employed in this study as the animal paradigm predictive of human psychosis as previously described by Johnson *et. al.* (1976). Mice were randomly allotted into various treatment groups (n = 6) and were given intraperitoneal (i.p.) injection of MJ (25, 50, 100 mg/kg), HLP (1 mg/kg) or vehicle (10 ml/kg). Sixty minutes later, each mouse received intraperitoneal (i.p.) injection of bromocriptine (5 mg/kg) and was placed immediately in a transparent observation chamber (20 cm × 20 cm × 23 cm). Thereafter, stereotype behaviours were observed for 2 min at 10, 15, 20, 30, and 45 after bromocriptine injection. Stereotype behaviours were scored as: 0 = absence of stereotype behaviour; 1 = presence of stereotype movements of the head; 2 = intermittent sniffing; 3 = chewing; 4 = intense licking.

3.5.2. Effect of MJ on Ketamine-induced stereotypy

Ketamine-induced stereotyped behaviour, a widely used animal model for psychosis, was further employed to screen for the antipsychotic effect of MJ in this study as previously described (Krystal *et. al.*, 1994; Yamamoto *et. al.*, 1997). Mice (n = 6) were given MJ (25, 50, or 100 mg/kg, i.p.), risperidone (0.5 mg/kg, i.p.), or distilled water (10 ml/kg, i.p.) 60 min before

induction of stereotyped behaviors with i.p. injection of ketamine (10 mg/kg). Each mouse was observed for stereotype behaviours in a transparent chamber (20 cm × 20 cm × 23 cm) for 2 min at time 10, 15, 20, 30, and 45 min, respectively. Stereotype behaviours were scored as described above.

3.5.3. Effect of methyl jasmonate on spontaneous motor activity (SMA)

The open field test was employed to screen the effect of MJ on SMA in mice. Mice (20–24 g, 6 per group) were given i.p injection of MJ (25, 50, 100 mg/kg), HLP (1 mg/kg), risperidone (0.5 mg/kg) or vehicle(1% Ethanol, 10ml/kg, i.p.) 60 min before each animal was placed in the centre of an open field chamber (72 cm×72 cm×36 cm). The number of lines crossed and duration of ambulation (s) for 5 min were recorded (Brown *et. al.*, 1999).

3.5.4. Effect of MJ on cataleptic behaviour

The cataleptic effect of MJ was investigated according to the modified version previously described by Costall and Naylor (1974). Mice (n = 6) were pretreated with i.p injections of MJ (25, 50, or 100 mg/kg), HLP (1 mg/kg), or distilled water (10 ml/kg) 60 min before testing for catalepsy. The test was done by gently placing the fore limbs of each animal on a horizontal plane wood surface (H = 6 cm; W = 4 cm; L = 16 cm) and the duration of akinesia (period of time the animal remained in one position, before initiating any active movement) in seconds was recorded.

3.5.5. Effect of MJ on Ketamine-induced hyperlocomotion

Ketamine-induced hyperlocomotion as measured by duration of ambulation (s) and frequency of lines crossed in the open field was also used to screen for antipsychotic-like effect of MJ (Yamamoto *et. al.*, 1997). The open field apparatus measuring 35 x 30 x 23 cm is made of a wooden wall and a frontal glass wall with visible lines which divides the floor of the chamber into 36 (20 x 20 cm) squares. Mice were randomly divided into six treatment groups (n = 6). Group 1 received vehicle (10 ml/kg, i.p.), while group 2-6 were treated with ketamine (20 mg/kg, i.p.), once daily for 14 days. From the 8th to 14th day of treatment, mice in group 2 were treated

with vehicle (10 ml/kg, i.p.), while group 3-5 mice received MJ (25, 50 and 100 mg/kg, i.p.) while mice in group 6 received risperidone (0.5 mg/kg, i.p.), 30 min after ketamine (20 mg/kg, i.p.) injection, respectively. Then, 24 h after the last treatment (day 15), each mouse was placed individually in the centre square of the open field chamber and allowed to explore freely. The duration of ambulation (s) and number of lines crossed were recorded for 5 min.

3.5.6. Effect of MJ on Ketamine-induced memory dysfunction

The effect of MJ on ketamine-induced memory dysfunction as measured by percentage alternation behaviour in the Y-maze was assessed as previously described (Monte *et. al*., 2013; Casadesus *et.al*., 2006). Y-maze consists of three identical arms labelled A, B, C and measuring 33 × 11 × 12 cm each, with the arms symmetrically separated at 120°. Mice were randomly divided into six treatment groups (n = 6). Group 1 received vehicle (10 ml/kg, i.p.), while group 2-6 were treated with ketamine (20 mg/kg, i.p.), once daily for 14 days. From the 8th to 14th day of treatment, mice in group 2 were treated with vehicle (10 ml/kg, i.p.), while group 3-5 mice received MJ (25, 50 and 100 mg/kg, i.p.) while mice in group 6 received risperidone (0.5 mg/kg, i.p.), 30 min after ketamine (20 mg/kg, i.p.) injection, respectively. Then, 24 h after the last treatment (day 15), each mouse was placed individually at the center of the Y-maze and allowed to explore all the three arms freely for 5 min. The number and sequence of arm entries were recorded and the apparatus was cleaned with 100% alcohol after each test. An entry was scored when the four paws of the animals were completely in the arm of the Y-maze. The percentage alternation, which is a measure of spatial working memory, was calculated by dividing the total number of alternations by the total number of arm entries, minus two and multiplied by 100 (Casadesus *et. al*., 2006). Alternation behaviour was defined as consecutive entries into all three arms (i.e. ABC, CAB or BCA but not BAB) for example (Casadesus *et. al.*, 2006).

$$\text{Degree of cognitive impairment} = \frac{\text{No of normal pattern}}{\text{No of arms entry - 2}} \times 100\%$$

3.6. Preparation of brain tissues for biochemical assays

After the behavioural tests, mice in the respective treatment groups, used for the 14 day experiment were sacrificed under ether anaesthesia and the brains were rapidly removed. Thereafter, each mouse brain obtained was weighed, homogenized with 10% w/v phosphate buffer (0.1M, pH 7.4) and centrifuged for 10,000 rpm at 4^{o}C for 15 min to obtain supernatants used for the biochemical assays.

3.6.1. Determination of brain level of malondialdehyde (MDA)

The brain level of MDA, a biomarker of lipid peroxidation, was estimated according to the method of Adam-Vizi and Seregi (1982). An aliquot of 0.4 mL of the supernatant was mixed with 1.6 mL of Tris–KCl buffer to which 0.5 ml of 30% TCA was added. Then, 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 min at 80°C. This was then cooled in ice and centrifuged at 3000 rpm for 15 min. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm using a spectrophotometer. The MDA concentration was calculated using a molar extinction coefficient of 1.56×105 M^{-1} cm^{-1} and values were expressed as µmoles of MDA per gram tissue.

3.6.2. Determination of reduced glutathione (GSH) concentration

Aliquots of brain supernatant of individual mouse in the respective treatment groups were taken and GSH concentration was determined using the method of Moron *et. al*. (1979). Equal volume (0.4 mL) of brain homogenate and 20% TCA (0.4 ml) was mixed and then centrifuged using a cold centrifuge at 10,000 rpm at 4°C for 20 min. The supernatant (0.25 mL) was added to 2 mL of 0.6 mM DTNB and the final volume was made up to 3 mL with phosphate buffer (0.2 M, pH 8.0). The absorbance was then read at 412 nm against blank reagent using a spectrophotometer. The concentrations of GSH in the brain tissues were expressed as micromoles per gram tissue (µmol/g tissue).

3.6.3. Estimation of superoxide dismutase (SOD) activity

The level of SOD activity was determined by the method of Misra and Fridovich (1972). Briefly, 0.1 mL of brain supernatant was added to 0.1 ml of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture, which was quickly mixed by inversion. The reference cuvette contained 2.6 mL buffer, 0.3 mL of adrenaline and 0.1 mL of distilled water. Then, increase in absorbance at 480 nm was monitored at 60 s intervals for 3 min. Superoxide dismutase activity was expressed as units of adrenaline consumed per minute per mg protein (Misra and Fridovich, 1972).

3.6.4. Determination of catalase activity

Catalase activity determined according to the method previously described (Sinha, 1971). Aliquots of mouse brain supernatant (0.1 mL) was added to 2 ml of sodium phosphate buffer (0.05 M; pH 7.4) and 0.9 ml of H_2O_2 (800 µmoles). The reaction mixture was mixed by a gentle swirling motion at room temperature and 1 mL of this mixture was added to 2 mL dichromate/acetic acid reagent. The absorbance was read using a spectrophotometer at a wavelength of 570 nm and change in absorbance at 60 s intervals. The catalase activity was expressed as µmol of H_2O_2 decomposed per minute per mg protein.

3.6.5. Protein estimation

Protein was measured in all brain supernatants for SOD and catalase estimation according to the method of Gornall *et. al*. (1949). Into a centrifuge tube is measure with Ostwald pippete, 0.5ml of Bovine serum and add 9.5mls of 22.6% Sodium sulfate. Stopper the tube and mix thoroughly by inversion (not by shaking).Transfer at once 2.0mls of the mixture into cuvette. Using a photoelectric colorimeter or spectrophotometer, transmitting maximally at 540mµ, adjust it to 100% transmission with the 'blank' cuvette in position. Replace blank with test cuvette and record the percentage transmission (or optical density) of each sample (Gornall *et al*., 1949).

3.7. Preparation of brain tissues for histology

After the behavioural tests, mice in the respective treatment groups, used for the 14 day experiment were anaesthetized with ether. Thereafter, 10% phosphate buffered formaldehyde was infused intracardially to perfuse the mice brain. The brains were then subjected to the routine method for paraffin wax embedment to obtain paraffin wax embedded tissue blocks. Transverse sections (5–6 µm thick) of the hippocampal region, striatum and substantia nigra were obtained with the aid of microtome (Leica, Germany) and the sections were fixed on glass slides (Hani *et al*., 2016)

3.7.1. Histology and estimation of neuronal density

Representative brain tissue sections of each treatment group were stained with Hematoxylin and Eosin to demonstrate general histology of the CA1 of the hippocampal region, striatum and substantia nigra region using the method of Eltony and Elgayar (2014). Thereafter, images were acquired using an Optronics Digital Camera connected to a computer interface (MagnaFire) and an Olympus BX-51 Binocular research microscope. The general structure of the pyramidal cell, peri-glomerular and granule cells were characterized using inter-reader variability. Viable neuronal cells were counted using Image J at X 960 at different microscopic fields for all groups using the method described by Eltony (2014). Viable neuronal cells were defined as round-shaped, cytoplasmic membrane-intact cells, without any nuclear condensation or distorted aspect. Neuronal density was calculated as a ratio of viable neuronal cell counts to square area of the circular view in a section.

3.8 Statistical analysis

All data are presented as Mean ± SEM. The results were analyzed by Kruskal-Wallis test, one way analysis of variance (ANOVA) and post hoc tests (Student's Newman–Keuls). These were carried out to determine the source of significant main effect using; Primer of Biostatistics software and Graph Pad InStat® Biostatistics software where appropriate. The level of significance for all tests was set at $\alpha_{0.05}$

CHAPTER FOUR

4.0 RESULTS

4.1 Effect of methyl jasmonate on stereotyped behaviours

Intraperitoneal injection of bromocriptine (5 mg/kg) or ketamine (10 mg/kg) produced stereotyped behaviours characterized by head movements, persistent sniffing, chewing, and intense licking in mice at 10, 15, 30 45, and 60 min after their administration. As in Figures 3 and 4, MJ (25, 50, 100 mg/kg, p.o.) significantly ($p < 0.05$) decreased these behavioural manifestations, suggesting antipsychotic-like activity. However, HLP (1 mg/kg, i.p.) or risperidone (0.5 mg/kg, i.p.) demonstrated greater inhibitory activity against stereotypy when compared with MJ (Figures 3 and 4).

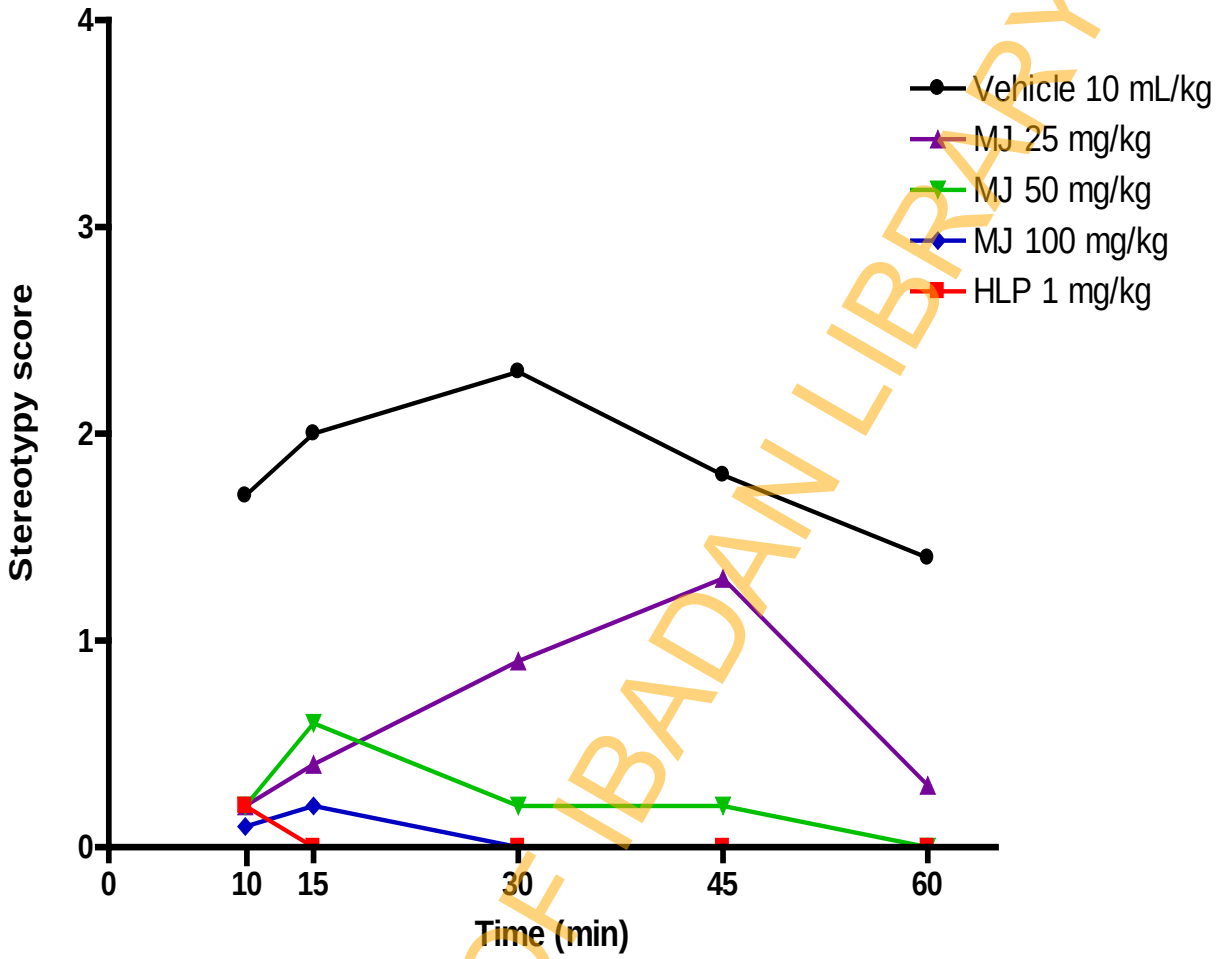

Figure 3: Effects of methyl jasmonate on bromocriptine-induced stereotyped behaviors in mice. Mice were treated with i.p injection of methyl jasmonate (MJ), haloperidol (HLP) or vehicle 60 min prior to i.p administration of bromocriptine and stereotypy was scored at different time intervals. Each point represents the mean score for 6 animals per group. SEM values are not provided since the data are not normally distributed. The Kruskal-Wallis test showed that there are significant ($^*p < 0.05$) differences between groups for each time interval.

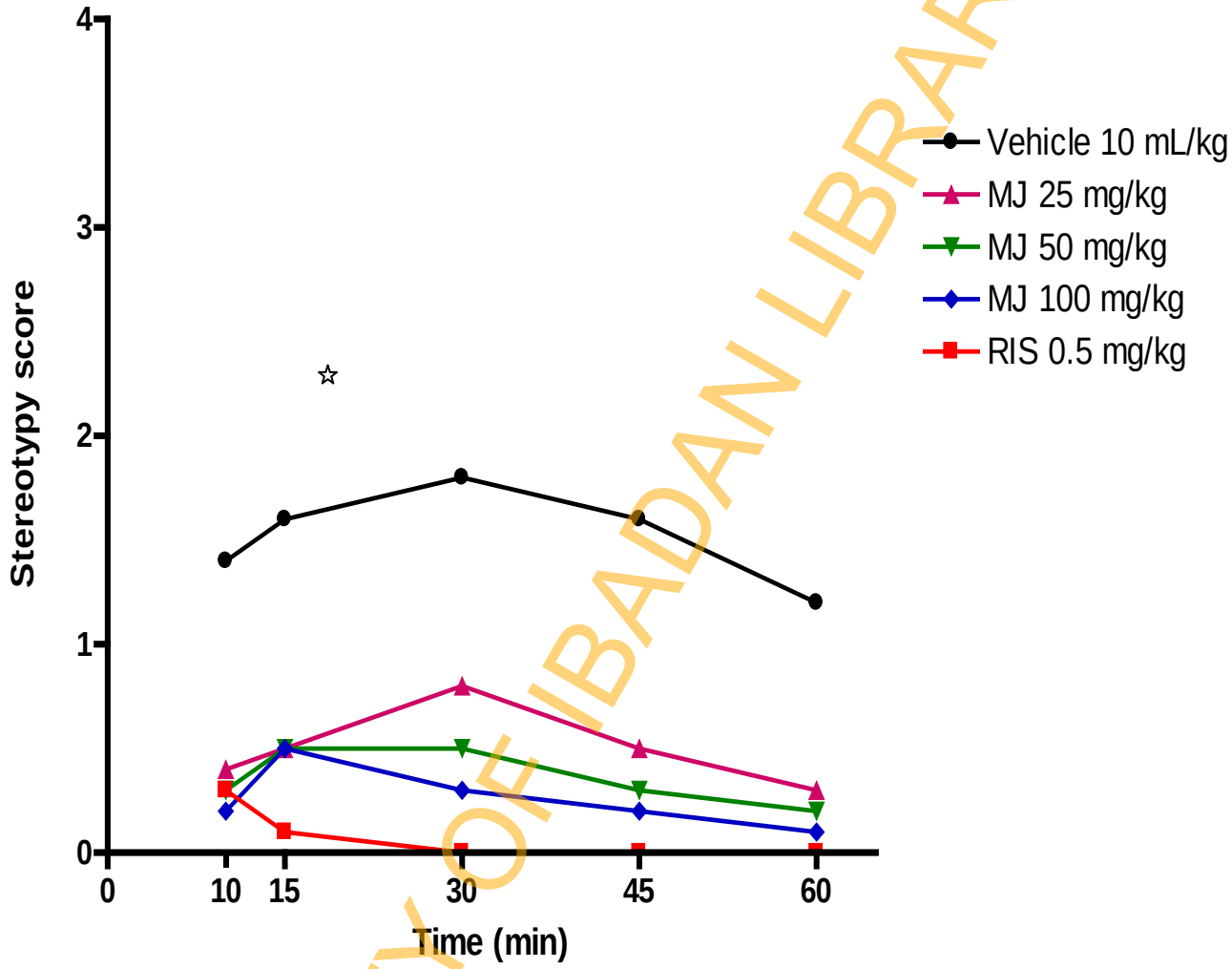

Figure 4: Effects of methyl jasmonate on ketamine-induced stereotyped behaviors in mice.

Mice were treated with i.p injection of methyl jasmonate (MJ), risperidone (RIS) or vehicle 60 min before acute injection of ketamine and stereotypy was scored at different time intervals. Each point represents the mean score for 6 animals per group. SEM values are not provided since the data are not normally distributed. The Kruskal-Wallis test showed that there are significant (*$p < 0.05$) differences between groups for each time interval.

4.2. Effect of Methyl Jasmonate on Spontaneous Motor Activity (SMA) and catalepsy

The effects of MJ on locomotion, an indication for sedation as measured by the number of lines crossed and duration of ambulation in the open field test are shown in Table 1. MJ (25, 50, 100 mg/kg, i.p.) did not significantly ($p > 0.05$) affect SMA, which suggest absence of sedative property (Table 1). As regards the effect on catalepsy, One-way ANOVA showed that HLP (1 mg/kg, i.p.) significantly ($p < 0.05$) prolonged the duration of akinesia in comparison with the vehicle-treated group (Fig. 5). In contrast, MJ (25, 50, 100 mg/kg, i.p.) did not significantly ($p > 0.05$) affect the duration of akinesia, which suggests absence of cataleptic effect in mice (Fig. 5).

Table 1: Effect of methyl jasmonate on Spontaneous Motor Activity in mice

Treatment	Dose (mg/kg)	Number of lines crossed	Duration of ambulation (s)
Vehicle	-	70.00±2.02	260.00±2.89
MJ	25	68.00±2.08	258.00±3.32
MJ	50	71.00±1.81	262.00±3.79
MJ	100	74.00±1.29	267.00±3.71
RIS	0.5	4.00±0.93*	38.00±3.56*

Mice were treated with i.p injection of methyl jasmonate (MJ), risperidone (RIS) or vehicle 60 min prior to the evaluation of SMA and the data were presented as the mean ± S.E.M. for 6 animals per group. *$p < 0.05$ compared with vehicle group (ANOVA followed by Newman–Keuls post hoc test).

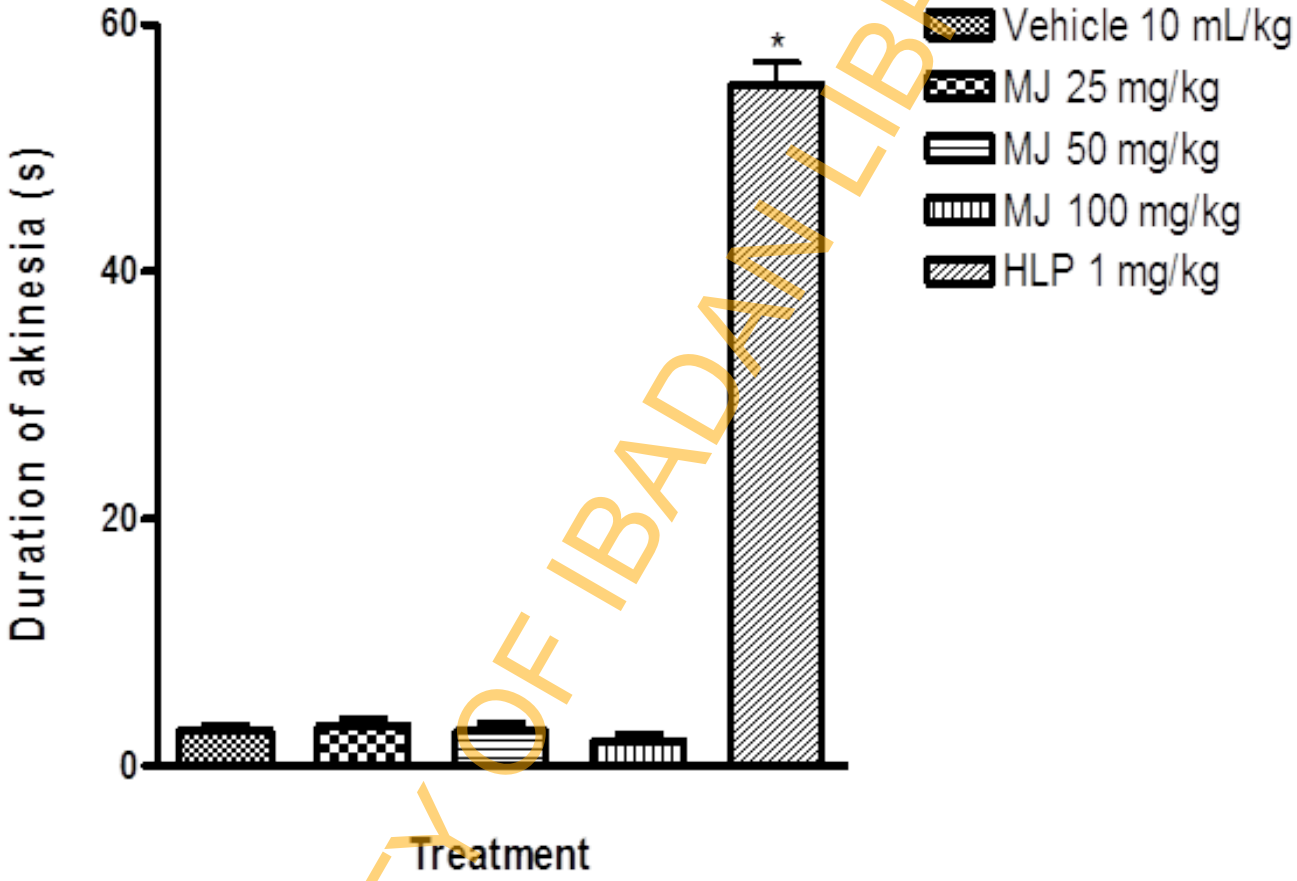

Figure 5: Effect of methyl jasmonate on the duration of akinesia in mice.

Mice were treated with i.p injection of methyl jasmonate (MJ), haloperidol (HLP) or vehicle 60 min prior to the evaluation of akinesia and the data were presented as the mean ± S.E.M. for 6 animals per group. $^{*}p < 0.05$ compared with vehicle group (ANOVA followed by Newman–Keuls post hoc test).

4.3. Effect of Methyl Jasmonate on ketamine-induced hyperlocomotion and cognitive deficit in mice

The effects of MJ on ketamine-induced hyperlocomotion and cognitive deficit in mice are shown in Table 2 and Figure 6. Ketamine (20 mg/kg, i.p.) produced a significant ($p < 0.05$) increase in hyperlocomotion (number of lines crossed and duration of ambulation) but significantly ($p < 0.05$) decreased memory performance (% alternation behaviour). However, MJ (25, 50, 100 mg/kg, i.p.) significantly ($p < 0.05$) reduced hyperlocomotion and also significantly ($p < 0.05$) reversed memory deficit induced by ketamine in mice (Table 2 and Fig. 6).

Table 2: Effect of methyl jasmonate on spontaneous motor activity in ketamine-induced stereotypy in mice

Treatment	Number of lines crossed	Duration of ambulation (s)
Vehicle 10 mL/kg	70.67±4.66	245.80±5.07
KET 20 mg/kg	185.00±3.63#	274.20±5.39#
MJ 25 mg/kg + KET	74.67±4.70	240.50±5.62
MJ 50 mg/kg + KET	75.67±2.88	243.70±4.90
MJ 100 mg/kg + KET	78.00±4.16	251.30±5.94
RIS 0.5 mg/kg + KET	54.83±2.99*	45.50±2.93*

Mice were treated with i.p injection of methyl jasmonate (MJ), risperidone (RIS), or vehicle 60 min prior to the evaluation of SMA, and the data were presented as the mean ± S.E.M. for six animals per group #$p < 0.05$ compared with vehicle group (ANOVA followed by Newman–Keuls post hoc test). *$p< 0.05$ compared with Ketamine treated group.

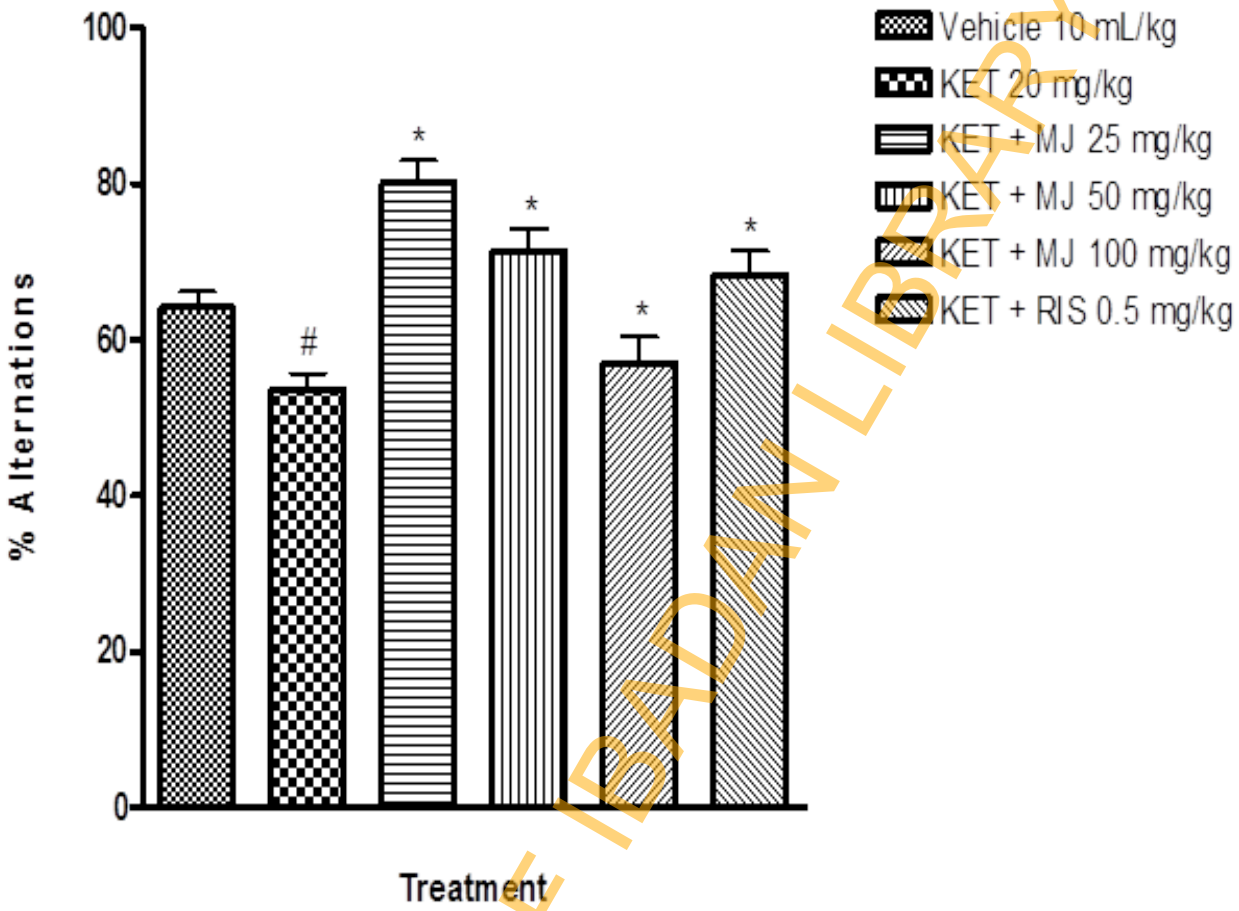

Figure 6: Effect of methyl jasmonate on chronic injection of ketamine-induced memory deficit in mice. Ketamine (KET), methyl jasmonate (MJ), risperidone (RIS). Values represent the mean ± S.E.M. for 6 animals per group. $^{\#}p < 0.05$ compared with vehicle group. $^{*}p < 0.05$ compared with ketamine group (ANOVA followed by Newman–Keuls post hoc test).

4.4. Effect of methyl jasmonate on ketamine-induced oxidative stress in mice brains

The effects of MJ on ketamine-induced changes in the activity of antioxidant enzymes (MDA, reduced GSH, SOD and catalase activities) in the mouse brain are presented in Table 3. Ketamine (20 mg/kg, i.p.) was found to significantly reduce MDA, GSH, SOD and catalase activities relative to vehicles ($p < 0.05$). However, pretreatment with MJ (25, 50 and 100 mg/kg, i.p.) or risperidone (0.5 mg/kg, i.p.) attenuated the effect of ketamine on the activity of these enzymes in a significant ($p < 0.05$) reduced manner.

Table 3: Effect of methyl jasmonate on the brain antioxidants in mice treated with chronic ketamine injection.

Treatment	GSH Concentration (µmol/g tissue)	MDA level (µmol/g tissue)	SOD activity (µmol/g tissue)	CAT activity (µmol/g tissue)
Vehicle 10ml/Kg	43.50±2.62	22.28±1.51	22.76±1.77	81.44±3.61
KET 20mg/Kg	21.95±2.69#	33.72±2.28#	13.08±1.33#	59.91±3.94#
MJ 25mg/Kg + KET	47.43±2.22*	19.96±1.64*	27.52±1.63*	86.63±4.65*
MJ 50mg/Kg + KET	42.23±2.83*	22.84±1.16*	24.41±1.49*	83.36±4.24*
MJ 100mg/Kg + KET	37.26±1.84*	24.65±1.70*	19.71±1.59*	76.06±3.22*
RIS 0.5mg/Kg + KET	39.56±2.46	23.21±1.33*	21.11±1.23*	80.33±3.64*

Values represent the mean ± S.E.M. for 6 animals per group. Ketamine (KET), Methyl jasmonate (MJ), risperidone (RIS). #$p < 0.05$ compared with vehicle group. *$p < 0.05$ compared with ketamine group (ANOVA followed by Newman–Keuls post hoc test).

4.5. Neuroprotective effect of methyl jasmonate in chronically ketamine-treated mice

Hematoxylin and Eosin (H&E) staining revealed neuropathological changes in the CA1 of the hippocampal region, striatum and substantia nigra of the brains of mice treated with ketamine (Plates 1, 2 and 3). In comparison with vehicle control group; with normal round shape and dark stained neurons, multiple pyknotic cells with evidence of distorted cytoarchitecture were observed in ketamine-treated mice brains. Furthermore, ketamine (20 mg/kg, i.p.) caused a significant ($p < 0.05$) decrease in the population of viable neuronal cells of the brain regions when compared with control, suggesting neurodegeneration (Figures 7, 8 and 9). However, administration of MJ (25, 50 and 100 mg/kg, i.p) attenuated neuropathological changes, as most of the neuronal cells recovered in their characteristic shapes when compared with the group treated with ketamine (Plates 1, 2 and 3). Also, MJ (25, 50 and 100 mg/kg, i.p) significantly ($p < 0.05$) attenuated the loss of neuronal cells of the CA1 of the hippocampal region, striatum and substantia nigra of the brains of mice treated with ketamine, which further suggests neuroprotective effect (Figures 7, 8 and 9).

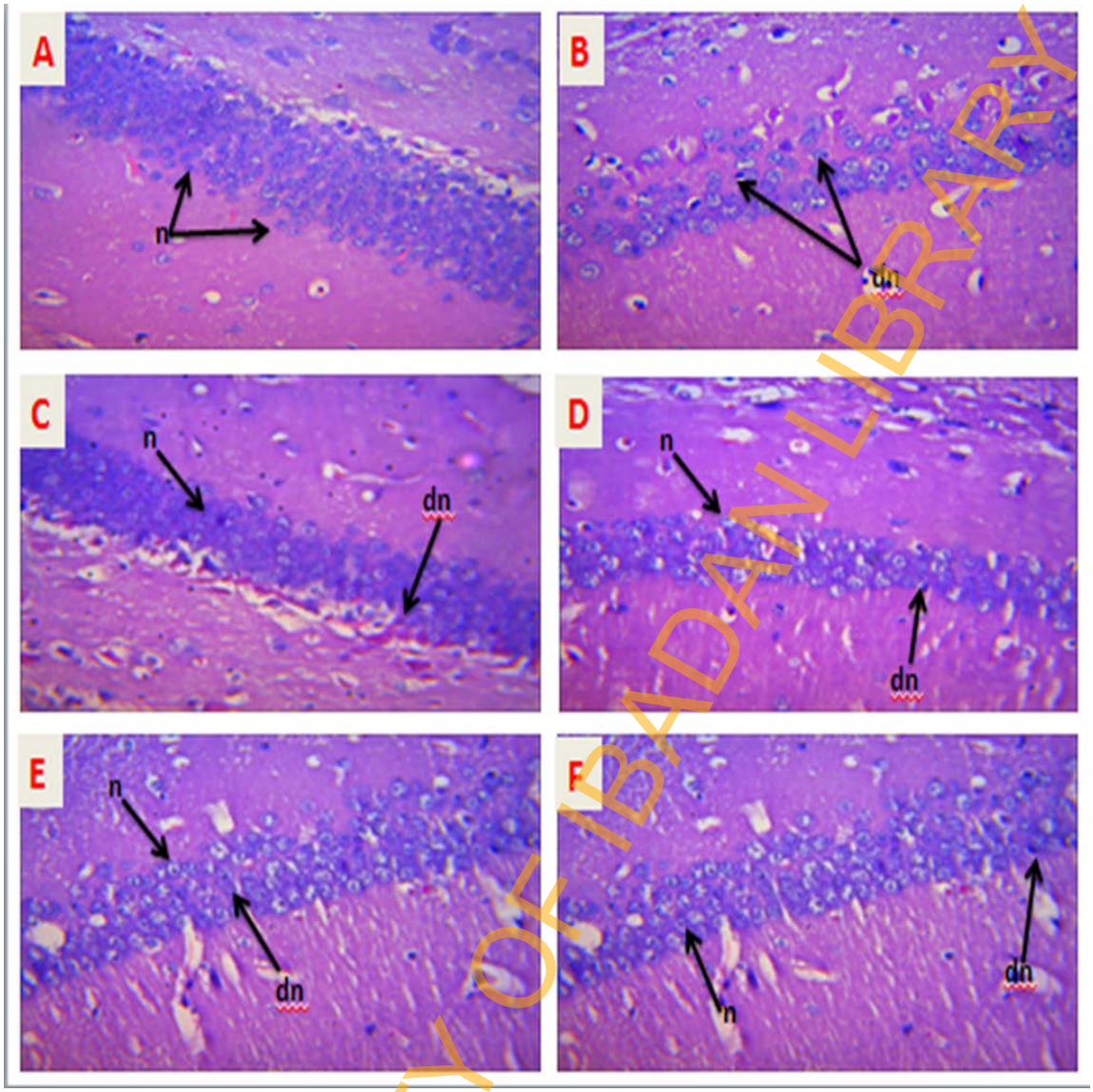

Plate 1: Representative stained section of hippocampus (CA1) of mice brain exposed to ketamine. Plates. (A) (control vehicle) shows normal histological patterns of cellular layers; (B) (Vehicle+Ketamine) shows degenerating neurons with distorted cytoarchitecture; (C) (MJ 25 mg/kg) shows normal pyramidal neuronal layers and cellular outline with some neurons exhibiting nucleoli and some degenerating neuron (D) (MJ 50 mg/kg) normal layers with few degenerating neurons; (E) (MJ 100 mg/kg) normal layers with few degenerating neurons; (F) (RIS 0.5 mg/kg) normal neurons with scanty degenerative changes. H&E×960 Normal neurons (n), degenerating neurons (dn) (RIS = Risperidone).

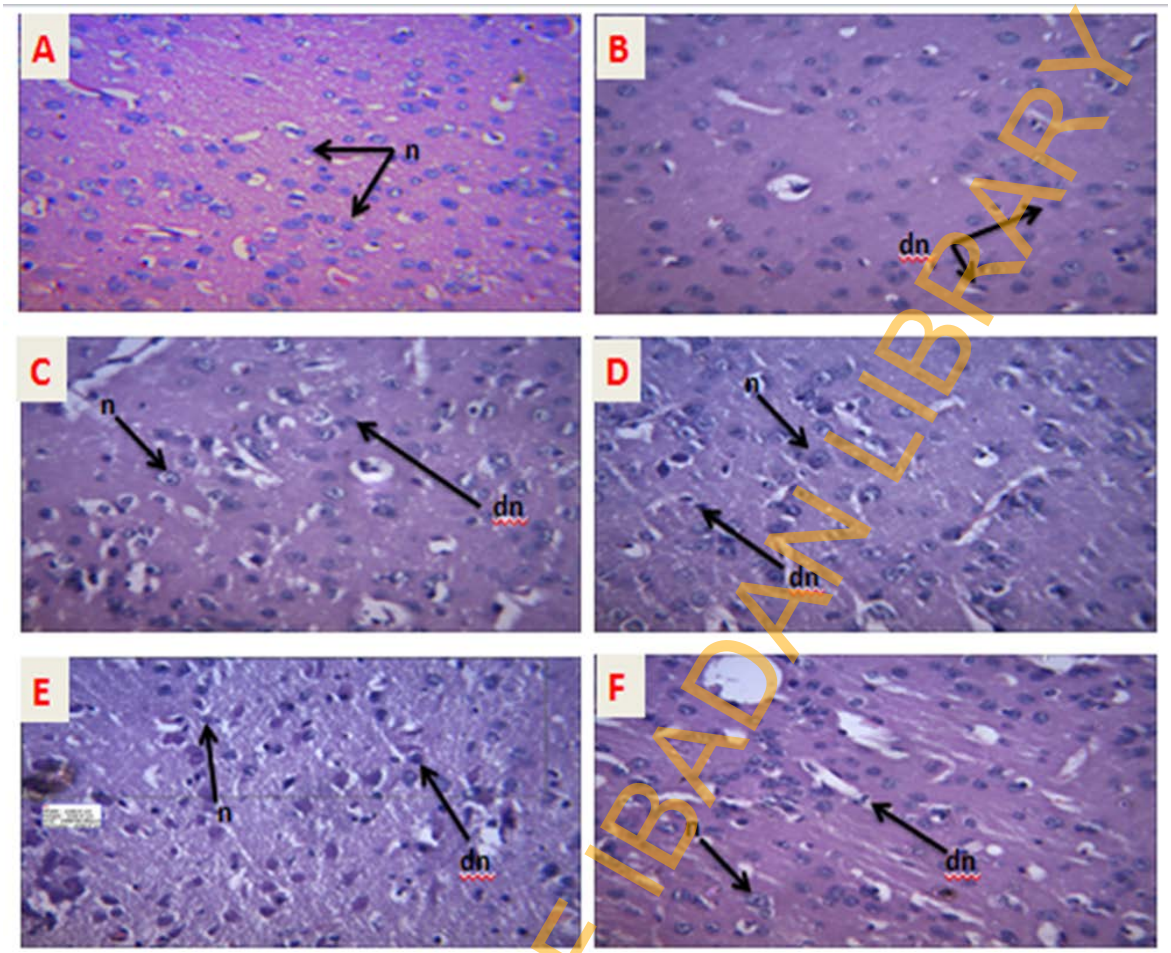

Plate 2: Representative stained section of striatum of mice brain exposed to ketamine.

Plates (A) (control vehicle) shows normal histological patterns of cellular layers; (B) (Vehicle+Ketamine) shows degenerating neurons with distorted cytoarchitecture; (C) (MJ 25 mg/kg) shows normal pyramidal neuronal layers and cellular outline with some neurons exhibiting nucleoli and some degenerating neuron (D) (MJ 50 mg/kg) normal layers with few degenerating neurons; (E) (MJ 100 mg/kg) normal layers with few degenerating neurons; (F) (RIS 0.5 mg/kg) normal neurons with scanty degenerative changes. H&E×960. Normal neurons (n), degenerating neurons (dn), (RIS = Risperidone).

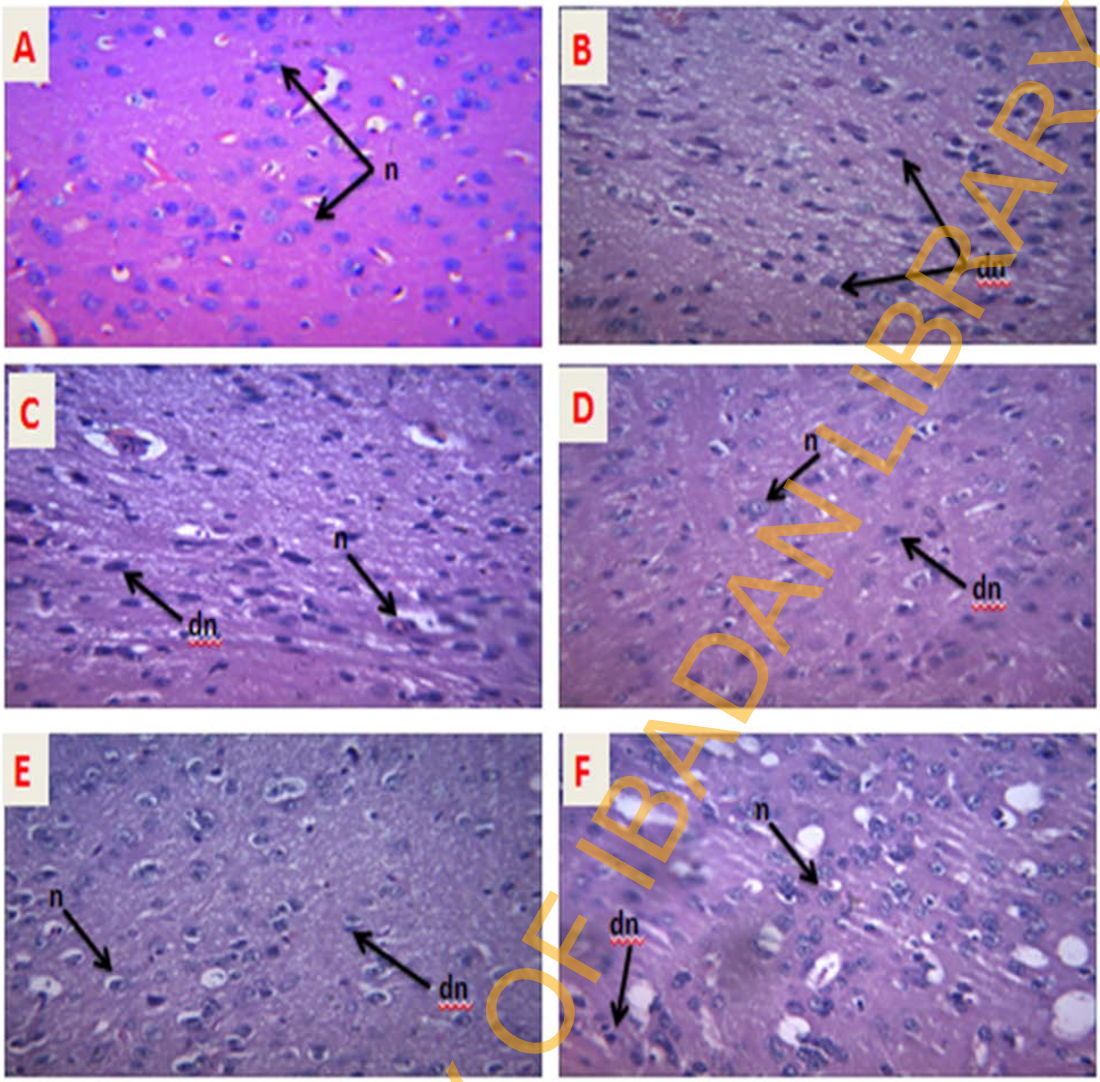

Plate 3: Representative stained section of substantia nigra of mice brain exposed to ketamine. Plates (A) (control vehicle) shows normal histological patterns of cellular layers; (B) (Vehicle+Ketamine) shows degenerating neurons with distorted cytoarchitecture; (C) (MJ 25 mg/kg) shows normal pyramidal neuronal layers and cellular outline with some neurons exhibiting nucleoli and some degenerating neuron (D) (MJ 50 mg/kg) normal layers with few degenerating neurons; (E) (MJ 100 mg/kg) normal layers with few degenerating neurons; (F) (RIS 0.5 mg/kg) normal neurons with scanty degenerative changes. H&E×960. Normal neurons (n), degenerating neurons (dn), (RIS = Risperidone).

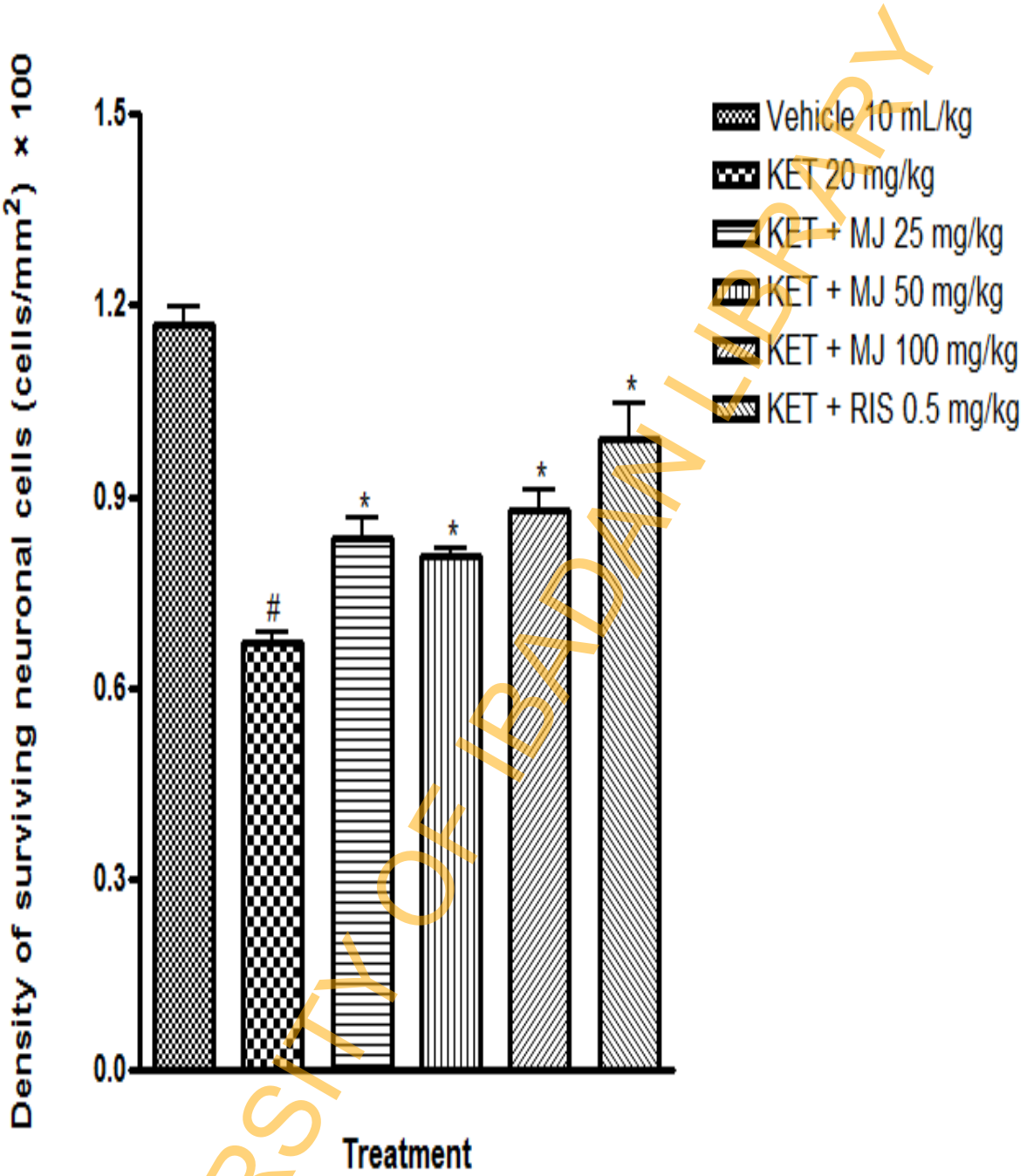

Figure 7: Effect of methyl jasmonate on the density of neuronal cells of cornu ammonis 1 (CA1) region of the hippocampus of mice induced by chronic ketamine injection. Each column represents mean ± S.E.M for 6 animals per group. $^{\#}P < 0.05$ compared with control. *$P < 0.05$ compared with ketamine group (ANOVA followed by Newman Keuls test).

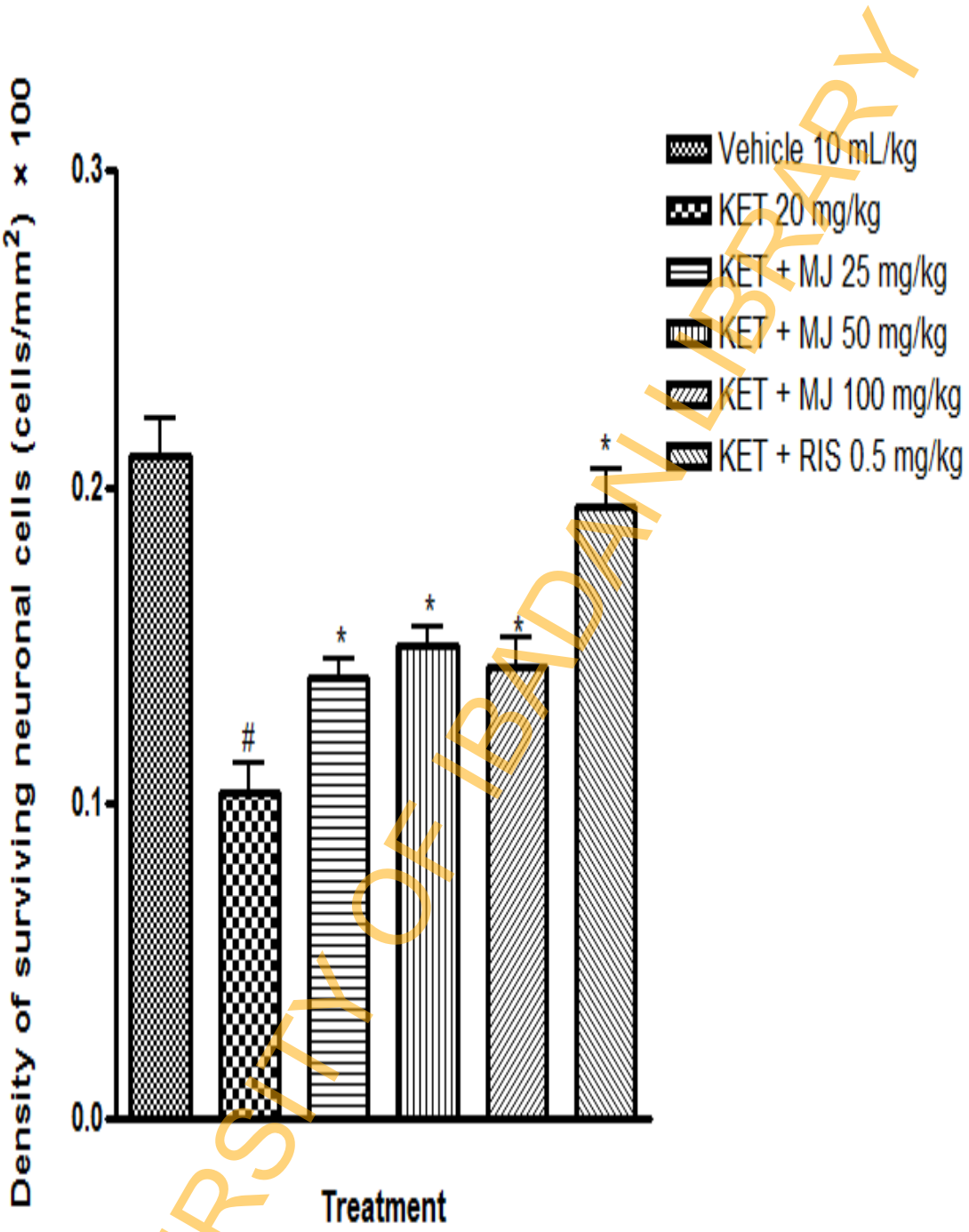

Figure 8: Effect of methyl jasmonate on the density of neuronal cells of the striatum of mice induced chronic ketamine injection in mice. Each column represents mean ± S.E.M for 6 animals per group). $^{\#}p < 0.05$ compared with vehicle group. $^{*}p < 0.05$ compared with ketamine group (ANOVA followed by Newman–Keuls post hoc test).

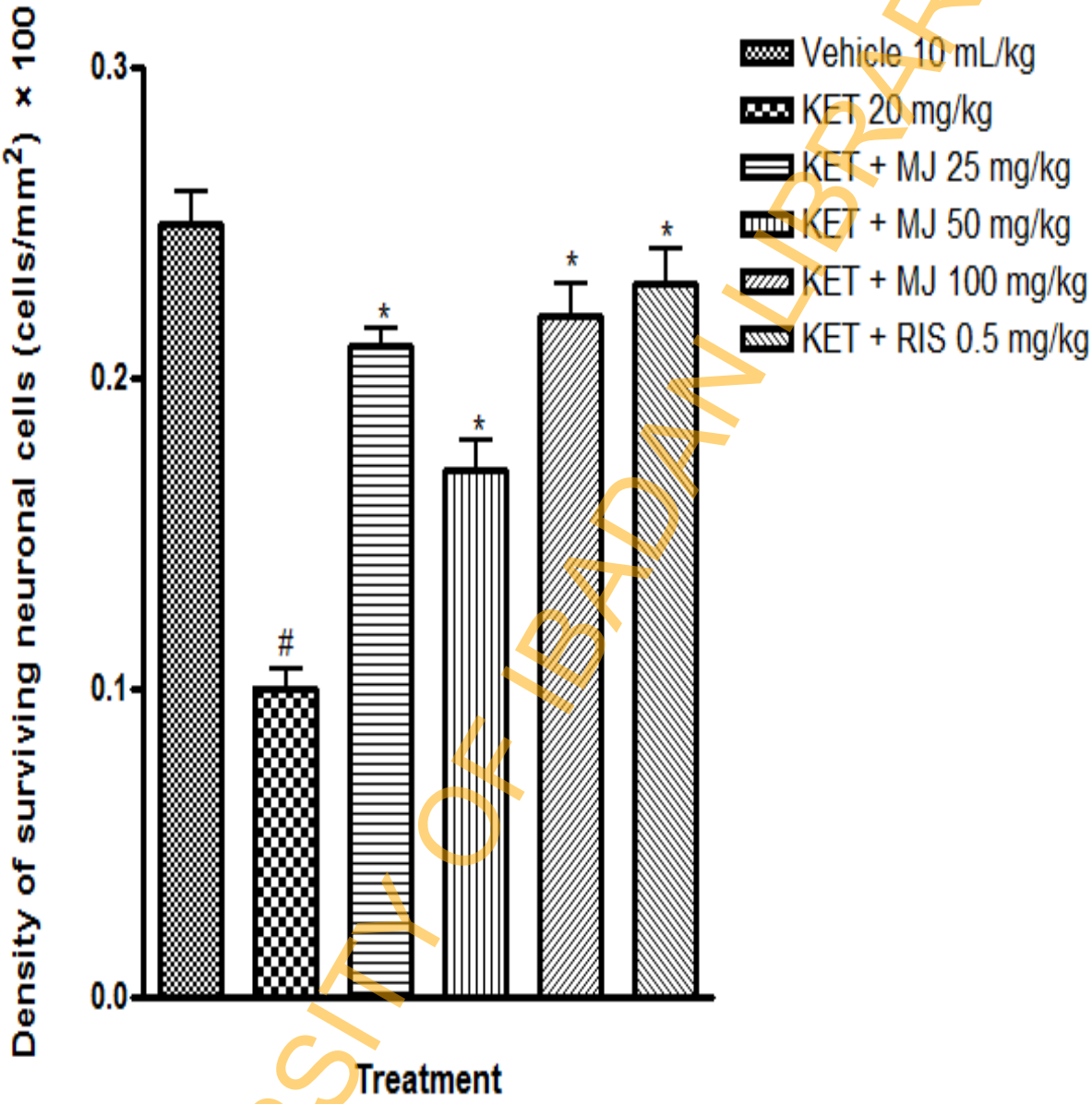

Figure 9: Effect of methyl jasmonate on the density of neuronal cells of the substantial nigra of mice induced by chronic ketamine injection. Each column represents mean ± S.E.M for 6 animals per group). $^{\#}p < 0.05$ compared with vehicle group. $^{*}p < 0.05$ compared with ketamine group (ANOVA followed by Newman–Keuls post hoc test).

CHAPTER FIVE

5.0 Discussion

The results of this study showed that methyl jasmonate produced a significant suppression of stereotyped behaviours induced by bromocriptine in a dose-dependent manner, which suggest antipsychotic-like activity. Stereotyped behaviour is one of the most prominent symptoms of psychosis and it usually manifest in humans in the form of repetitive performance of a set of strange gestures or asking the questions or making the same kind of comments and it is susceptible to the blocking effect of antipsychotic drugs (Davies *et. al.*, 1991). The antagonism of stereotyped behaviours induced by bromocriptine has served as an additional animal model for screening novel compounds with antipsychotic property (Bourin *et. al.*, 1986). The neurochemical pathway involved in the mediation of bromocriptine-induced stereotyped behaviours has been shown to be related to dopamine, a neurotransmitter implicated in the pathophysiology of psychotic disorders (Alan-Boyd, 1995). Specifically, studies have shown that stereotypy induced by bromocriptine is mediated via direct stimulation of dopamine receptors. Bromocriptine has been shown to replicate all the features produced by conventional dopaminergic agents like apomorphine or amphetamine. Although its stereotypic effect is less intense and the dose response curves are flatter but after a delay in the onset of action, its durations of action are considerably longer. Bromocriptine also causes turning contralateral to the lesions opposite to the way an injection of 6-hydroxydopamine into the substantia nigra and the subsequent denervation hypersensitivity developed at dopaminergic receptors on the lesioned side like amphetamine (Johnson and Loew, 1976). Thus, the findings that Methyl jasmonate (MJ) suppressed stereotyped behaviours induced by bromocriptine in mice in a similar manner to haloperidol, suggest that it might offer beneficial effect in the alleviation of certain symptoms of psychotic disorders.

The antipsychotic property of MJ was further evaluated using ketamine-induced stereotyped behaviors in mice. Ketamine produces stereotypy through antagonism of N-Methyl-D-aspartate (NMDA) receptor that is involved in inhibition of mesolimbic dopamine release. Ketamine acts through a noncompetitive blockade of NMDA-glutamate receptor (Duncan *et. al.*, 1998). Low dose of Ketamine causes excitatory effects after absorption into the systemic circulation, and this has been ascribed to either disruption of the negative feedback regulation of excitatory amino-

acid secreting neurons, or from disinhibition of neuronal activity (Duncan *et. al.*, 1998). More specifically, the increase in stereotypy scores induced by ketamine has been indirectly ascribed to activation of dopaminergic pathways (Kapur, 2003). The stereotypy is due to blockade of NMDA-receptors located on inhibitory Gamma-Amino-Butyric Acid neural system(GABAergic) neurons in the limbic and sub-cortical brain regions and thus the release of the inhibitory action of GABAergic system has been shown to be responsible for the release of dopamine and serotonin in the brain (Allan and Robert, 2005). However, antipsychotic drugs are known to inhibit the stereotypy behavior produced by ketamine. The results of this study showed that MJ significantly decreased these behavioural disorders elicited by i.p. injection of ketamine, which further suggest antipsychotic-like property in mice.

The antagonism of hyperlocomotion induced by chronic administration of ketamine is a well-recognized animal model routinely used for evaluation of compounds with antipsychotic activity (Yamamoto *et. al.*, 1997). The neurochemical mechanism underlying ketamine-induced hyperlocomotion or hyperactivity when administered chronically is closely connected with the activation of glutamaternergic receptors located in the limbic region of the brain (Yamamoto *et.al.*, 1997). In this study, chronic ketamine administration produced increase in locomotion in mice, which was inhibited by MJ in a dose-related manner in an open field test. Hyperactivity produced by ketamine in rodents is akin to psychotic agitation or excited catatonia seen in patients with schizophrenia (Brown *et. al.*, 1999). Hyperactivity or agitation and displays of aggressive behaviors are integral components of psychomotor excitement commonly seen in most patients with psychotic disorders (Brown *et. al.*, 1999). Patients in the state of psychomotor excitement constitute sources of danger to themselves and others, thus requiring the use of drugs with a tranquillizing effect such as antipsychotic medications (Yamamoto *et. al.*,1997). The findings that MJ inhibited ketamine-induced hyperlocomotion activity further indicate its potential utility as an antipsychotic agent.

The clinical manifestations of psychotic disorders have been classified as positive, negative and cognitive symptoms (Chatterjee *et al.*, 2012; Kukla and Gold, 1999). Bromocriptine, amphetamine and apomorphine models are known to replicate the positive symptoms of schizophrenia (Johnson *et al.*, 1976; Sams-Dodd, 1998; Chatterjee *et al.*, 2012) whereas chronic

ketamine injection can replicate both the positive, negative and cognitive symptoms associated with the disease (Chatterjee et al., 2012). However, acute dose of ketamine according to literature does not induce the negative and cognitive symptoms associated with schizophrenia (Chatterjee et al., 2012). Cognitive dysfunction in particular is very important as it contributes to the poor quality of life of patients with the disease (Draper *et al.*, 2009; Lesh *et al*., 2011; Barch and Sheffield, 2014). Moreover, regular use of the currently available antipsychotic drugs offers little or no benefits for psychotic patients with cognitive impairment (Draper *et al*., 2009; Lesh *et al*., 2011; Barch and Sheffield, 2014). The ability of MJ to improve cognitive functions in psychotic disorders was assessed in mice chronically treated with ketamine using Y-maze paradigm. Y-maze test is a well-recognized animal model used routinely as an alternative to Morris water maze for screening compounds with memory enhancing effect in rodents (Blokland *et. al*., 2005). The Y-maze test does not involve active swimming and is therefore considered to be less anxiogenic than the Morris water maze. The Y-maze test is based on the ability of rodents to remember the sequence of arms entry, commonly known as spontaneous alternations (Blokland *et. al.*, 2005). Thus, the list of arms visited is believed to be held in spatial working memory, thereby preventing revisits to the previous arm. Rodents always remember the last arm visited in order to alternate the arm choice, thus serving as a measure of short-term working memory (Lee *et. al.*, 2010). The results of this study showed that ketamine produced a significant decrease in alternation behaviour, which suggest cognitive dysfunction. Memory impairment caused by ketamine is known to be related to antagonism of glutaminergic neurotransmission, as well as induction of oxidative stress in the brain. Acetylcholine, the neurotransmitter that play crucial role in memory has been found to be reduced following ketamine injection (Blockland *et. al*., 2005). Increased activity of acetylcholinestersase enzyme in the brain especially in the hippocampal region further lends credence to ketamine-induced memory deficifits. Also, ketamine has been reported to antagonize nicotinic acetylcholine receptor that regulates glutamate release. The blockade of this receptor may also contribute to the dfmemory impairing effects of ketamine. The finding that MJ significantly reversed in a dose-dependent manner the cognitive dysfunction induced by ketamine suggest it might be beneficial in psychotic patients with memory deterioration.

Oxidative stress has been implicated in the pathophysiology of several neuropsychiatric disorders, including psychosis (Machado-Vieira *et. al*., 2007; Selek *et. al.*, 2008). The results of this study showed that ketamine induces changes in some oxidative stress parameters like increase in MDA levels and decrease in the antioxidant defense system. Previous studies have confirmed that the behavioural changes produced by ketamine are associated with the induction of oxidative stress in different brain regions (Brocardo *et. al.*, 2010; Ghedim *et. al*., 2012). It is important to highlight that brain the brain is known to be more vulnerable to oxidative damage for several reasons. These include its high metabolic activity, low levels of protective antioxidant enzymes, high proportion of readily oxidizable membrane polyunsaturated fatty acids, auto-oxidizable neurotransmitters (dopamine, adrenaline and noradrenaline) and presence of redox-catalytic metals (iron and copper) (Halliwell, 2006). Additionally, the brain is also susceptible to secondary and self-perpetuating damage resulting from oxidative cellular injury or neurosis, via the neurotoxic effects of released excitatory amines (mainly glutamate) and activated inflammatory cells (Halliwell, 2006). Metabolism of neurotransmitters generates large amounts of hydrogen peroxide (H_2O_2) and neuronal mitochondrial can generate superoxide radical (Mahadik and Mukherjee, 1996; Halliwell, 2006). However, hippocampal neurons are known to be more vulnerable to oxidative stress and this may contribute significantly to cognitive impairing effect of ketamine (Lowenstein *et. al*., 1992; Zhao and Flavin, 2000; Golarai *et. al*., 2001; Candelario-Jalil *et. al*., 2001; Geddes *et. al.*, 2003). Furthermore, H&E) staining revealed the presence of neuropathological changes in the CA1 of the hippocampal region, striatum and substantia nigra of the brains of mice treated with ketamine. In addition, ketamine (20 mg/kg, i.p.) caused a significant ($p < 0.05$) decrease in the population of viable neuronal cells of the brain regions when compared with control, which suggest neurodegeneration. However, administration of MJ reuced increased oxidative stress and attenuated neuropathological changes, as most of the neuronal cells recovered in their characteristic shapes when compared with the group treated with ketamine. Also, MJ significantly attenuated the loss of neuronal cells of the CA1 of the hippocampal region, striatum and substantia nigra of the brains of mice treated with ketamine, suggesting neuroprotection. Indeed, in recent years, several studies have highlighted the ability of MJ to reduce oxidative stress and neurodenegeration produced by lipopolysaccharide and scopolamine in animal models of cognition (Eduviere *et. al*., 2015; Eduviere *et. al*., 2016). Besides that, MJ administration demonstrated adaptogenic property in

mice exposed to unpredictable chronic mild stress via suppression of increased oxidative stress and protection of neuronal cells against the damaging effects of unpredictable chronic mild stress in mice (Umukoro *et. al*., 2016). Although, more studies are needed before concluding on the mode of action of MJ, the present data suggest that its antipsychotic effect may be related to suppression of dopaminergic activity, oxidative stress and neurodenegeration.

The induction of catalepsy in animals by typical neuroleptic drugs like haloperidol is believed to be due to inhibition of dopaminergic neurotransmission (Van Rossum *et. al*., 1970). Thus, the test for catalepsy, as a paradigm to detect the tendency of antipsychotics to induce extra pyramidal adverse effects based on the prolongation of the duration of akinesia is routinely carried out in rodents. However, in this study, the antipsychotic-like activity demonstrated by MJ was not associated with cataleptic behaviour in mice, which suggest that its effect may be devoid of extra pyramidal adverse effects.

CHAPTER SIX

6.0 SUMMARY AND RECOMMENDATION

6.1. SUMMARY

This study provides valuable evidences, which suggest that methyl jasmonate demonstrated antipsychotic-like property and ameliorated cognitive deficit produced by ketamine in mice. The improvement of memory function makes MJ a potential agent that may be used therapeutically for management of psychotic patients with cognitive deteriorations that could lead to better quality of life outcomes. It is speculated that its mode of action may involve antioxidation, neuroprotection and interference with dopaminergic neurotransmission.

6.2. CONTRIBUTION TO KNOWLEDGE

- The study has provided preclinical data which suggest the potential usefulness of methyl jasmonate in the treatment of patients with psychotic disorders.
- The study also revealed the potential ability of methyl jasmonate as an agent that may be used therapeutically to ameliorate cognitive deteriorations commonly associated with this disease thereby improving the quality of life of patients with psychosis (as the memory improves).
- This study also provide experimental data that showed that anti-oxidation and interference with dopaminergic neurotransmission may underlie the antipsychotic-like effect of methyl jasmonate

6.3. RECOMMENDATIONS FOR FURTHER STUDIES

•Evaluation of the effect of MJ on inflammatory cytokines in ketamine-treated animals

•Since H&E staining can only provide a rough estimate of the population of viable cells, immunocytochemistry techniques that can quantify viability of neuronal cells is recommended for further evaluation of the neuroprotective effect of MJ in ketamine model of psychosis.

•Evaluation of the effects of MJ on ketamine withdrawal psychosis episodes that may provide information on relapse episodes

•Assessment of the effect of MJ on the brain concentrations of neurotransmitters like dopamine, serotonin and glutamate. Radioligand binding assays may also be necessary to clearly reveal the interaction of MJ with receptor systems suggestive of antipsychotic property.

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