INVESTIGATION OF ANTIPSYCHOTIC ACTIVITY AND TOXICITY PROFILE OF *TAPINANTHUS DODONEIFOLIUS* MISTLETOE GROWING ON *PARKIA BIGLOBOSA* IN MICE AND RATS

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NOVEMBER, 2019

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B. Pharm (ABU) 2014
P16PHPG8078

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

DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS,
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ZARIA, NIGERIA

NOVEMBER, 2019

DECLARATION

I declare that the work in this Dissertation entitled 'Investigation of Antipsychotic Activity and Toxicity Profile of *Tapinanthus dodoneifolius*Mistletoe Growing on *Parkia biglobosa* in Mice and Rats'' has been performed by me in the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The information derived from the literature has been duly acknowledged in the text and a list of references provided. No part of this Dissertation was previously presented for another degree or diploma at this or any other Institution.

Vivian MAMMAN _____ _____

Signature **Date**

CERTIFICATION

This dissertation entitled "INVESTIGATION OF ANTIPSYCHOTIC ACTIVITY AND TOXICITY PROFILE OF *TAPINANTHUS DODONEIFOLIUS* MISTLETOE GROWING ON *PARKIA BIGLOBOSA* IN MICE AND RATS'' by Vivian Mamman, meets the regulations governing the award of the degree of Masters of Science in Pharmacology of the Ahmadu Bello University, and is approved for its' contribution to knowledge and literary presentation.

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Chairman, Supervisory Committee Signature Date

Dr. M.G. Magaji _____ _____
Member, Supervisory Committee Signature Date

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Dean, School of Postgraduate Studies Signature Date

DEDICATION

I dedicate this dissertation to late Abubakar Yusuf (Kojo)

ACKNOWLEDGMENT

I thank God Almighty who guided, protected, inspired, motivated and strengthened me throughout the course of this research work. In spite of all the challenges encountered, God nourished me with all the resources, good health, wisdom, knowledge and willpower that made my study a success. Words are too short to express my gratitude.

My profound and deep gratitude goes to my supervisors; Prof N.M. Danjuma and Dr. M.G. Magaji. Despite your busy schedules, I sincerely appreciate your constant encouragement, patience, tolerance, advice, suggestions and corrections that helped in standardizing and accomplishing this work. It is indeed a great pleasure and honour working under your supervision and acquiring so much knowledge and experience.

I am grateful to all the lecturers of the Department of Pharmacology and Therapeutics whose impact had helped to build me up both in character and in learning. To my academic mentors, Dr. Aisha Shehu, Mallam Abdullahi Balarabe Nazifi and Pharm Bukhari Mahmud, I am immensely grateful for all your supports. I also warmly and gratefully acknowledge the timely assistance and readiness to help attitudes of the entire administrative, technical and Animal House Staff of the Department of Pharmacology especially, Mal Abubakar Balarabe, Mal Muazu, Mal Bashir, Baba and Mal Idris for providing a conducive and friendly environment and facilities that enhanced the success of this research work. Not forgetting the assistance of Department of Pharmacognosy Laboratory Staff (especially Mal M. Kabiru) and Department of Anatomy Laboratory (especially Mr. A. Bamidele).

I will not forget to thank all the staffs of General Hospital Kachia, most especially Pharm J. Turman and Pharm J.A. Waziri for their support and patience during the course of my study.

My deepest love and gratitude to my beloved family, for their support; both financial and morale, love and prayers and for believing in me. May the good Lord preserve you all

I remember and cherish with gratitude my coursemates and friends especially Baba, David, Amanda, Abraham, Monica, Atiku, Patrick, Alasirin, Senator, Helen, Halima, Yakubu, Fatima, Isaiah, just to mention but a few for their love, support, encouragement, best wishes, prayers as well as good company outside study/lab environment to help ease the stress of academic work. Thank you good friends and remain blessed.

Finally, I still give praise to my Heavenly Father, God most High who has made everything possible through His everlasting love and abundant grace.

ABSTRACT

Tapinanthus dodoneifolius (Loranthaceae) has diverseethnomedicinal use which include the management of mental illness, epilepsy, diarrhoea, malaria, stomach pain, dysentery and hypertension.Despite these uses and many validated pharmacological properties, the antipsychotic effect and toxicity profile of *T. dodoneifolius* has not been established. The aim of the study was to establish the pharmacological rationale and sub-chronic toxicity profile for the ethnomedicinal use of *T. dodoneifolius* in the treatment of mental illness. The methanol whole plant extract of *Tapinanthus dodoneifolius* (METD) was prepared. Phytochemical screening of the methanol extract of *T. dodoneifolius* was carried out using standard protocols. The oral median lethal dose (LD_{50}) was estimated. Sub-chronic toxicity study was also carried out by administering the extract to rats at doses of 250, 500 and 1000 mg/kg for 28 consecutive days. General pharmacological study using beam walking assay was carried out. The antipsychotic activity (model for efficacy) of the METD(250, 500, 1000 mg/kg) was investigated using apomorphine-induced stereotype climbing behaviours; swim-induced grooming; ketamine-induced hyper-locomotion; ketamine-enhanced immobility in forced swim test; ketamine-induced schizophrenic-like behaviour while catalepsy and vacuous chewing movement tests was carried out to investigate side effects of (METD). The Phytochemical screening revealed the presence of alkaloids, cardiac glycosides, saponins, tannins, flavonoids, steroids, terpenoids and carbohydrate. The LD_{50} was estimated to be greater than 5000 mg/kg. In the sub-chronic toxicity study, the extract showed significant($p \leq 0.05$) increase in body weight and decrease in alkaline phosphatase (ALP) but no significant difference in the kidney function test, haematological analysis and relative organ weight of selected organs. The histology of the heart revealed normal cardiac muscles. However, the histology of the kidney showed a non-dose dependent histological

changes (slight tubular necrosis with lymphocyte hyperplasia). The highest dose of the METD (1000mg/kg) on the liver, showed a moderate hepatocyte necrosis while the 250 mg/kg and 500 mg/kg showed a normal hepatocytes. A dose dependent pathological changes (slight alveoli congestion, nuclei hardening and pyknosis) were seen in the histology of the lungs of the METD. A normal red and white pulp was observed at 250 mg/kg however, lymphocyte hyperplasia was seen at doses of 500 and 1000 mg/kg of treated group extracts). Similarly, normal gastric mucosa was observed at 250 mg/kg and 500 mg/kg treated groups while lymphocyte hyperplasia in rats treated with METD 1000 mg/kg was seen. The METD at all doses tested produced an insignificant increase in the number of foot slips in mice for beam walking assay. In apomorphine-induced stereotype climbing**,** METD produced a non-significant dose-dependent decrease in mean climbing behaviour in mice while for swim-induced grooming, METD at all doses produced a significant ($p \leq 0.05$) dose-dependent decrease in mean grooming time in mice. In ketamine-induced hyper-locomotion, METD produced a non-significant dose dependent decrease in number of line crossing and a significant ($p < 0.01$) increased in immobility time in mice. METD at all the doses significantly ($p \leq 0.05$) decreased immobility time induced by ketamine in forced swim test. In ketamine induced schizophrenic-like behaviour, METD (1000mg/kg) non-significantly decreased the number of lines crossed in the open field test and non-significantly increased percentage correction alternation in mice.METD non-significantly increased the social preference time in social interaction test. METD non-significantly increased the duration of catalepsy and the number of vacuous chewing movement in rats. These results suggest the presence of pharmacologically active

constituents in the METD, with antipsychotic effects and does not seem to possess the toxic effects that could affect its ethnomedicinal uses in the management of mental illnesses.

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ABBREVIATIONS, GLOSSARY AND SYMBOLS

ABU - Ahmadu Bello University

ALB- Albumin

ALP - Alkaline phosphatase

ALT - Alanine aminotransferase

ANOVA - Analysis of variance

APA- American psychiatric association

AST - Aspartate aminotransferase

CNVs- Copy number variants

DSM- Diagnostic and statistical manual of mental disorder

FMRI- Functional magnetic resonance imaging

g - Grams

g/dL- Milligram per deciliter

GAD- Glutamic acid decarboxylase

Gran- Granulocytes

Hb - Haemoglobin

HCT- Haematocrit

HIV -Human immunodeficiency virus

ICD- international statistical classification of disease and related health problem

Ig- Immunoglobulin

i.p - Intraperitoneal

IU/L - International units per Litre

LD_{50} - Lethal dose in 50% of population

LYMPH- Lymphocytes

mEq/L - Milliequivalents per litre

mg/dL - Milligram per decilitre

mg/kg - Milligram per kilogram

mmol - Millimoles

mmol/L - Millimoles per litre

METD- Methanol whole plant extract of *Tapinanthus dodoneifolius*

MON- Monocytes

n - Number of animals in a group

OECD - Organisation of Economic Cooperation and Development

< - Less than

≤ - Less than or equal to

P - Probability

PET- Positron-emission tomography

p.o - Per Orally

RBC - Red blood cell

SEM - Standard error of mean

WBC - White blood cell

WHO - World Health Organisation

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Schizophrenia is a psychological disorder characterized by psychotic symptoms such as hallucinations and delusions, which significantly affect emotions, behaviour and most notably, mental processes and mental contents (Hodgins, 1992). Psychosis is a condition of mental illness that causes a person to lose his or her sense of reality. People with this disease have delusions, hallucinations, disorganized speech, grossly disorganized behaviour, or catatonic behavior (Ahmed and Azam, 2014).Schizophrenia is one of the top five causes of disability among adults in developed nations, with heart disease, arthritis, drug use, and HIV (Murray and Lopez, 1996).

Herbal medicine also called botanical medicine or phytomedicine refers to using a plant's seeds, berries, root, leaves, bark or flowers for medicinal purposes and is the oldest known form of medicine(Krishna, 2012). It has been used for over 2,000 years and is still the major form of medicine for over 75% of the world's population (Krishna, 2012). In Nigeria and most developing countries of the world, rural and urban dwellers, literate or illiterate rely heavily on herbal preparations for the treatment of various diseases despite the availability of orthodoxmedicine (Nwabuisi, 2002).

In some parts of the world, various plants and plant parts are often used in combination with different rituals for treatment of mental disorders (Magaji *et al.,* 2008; Ahmed and Azam, 2014).

Our ancestors used trial and error to discover the most effective local plants for the treatment of illnesses. Advances in science have enabled a better understanding of the

pharmacologicaleffects of herbs on the human body and therefore their role in restoring health. During the course of history the cure of disease and the use of medicinal plant have been much influenced by religious practice and exercise of magical rites (Kokate, 2001).

Medicinal plants contain various phytochemicals and these phytochemicals can play an important role in reducing occurrences of many diseases by boosting various organ functions of the human body (Gilani and Atta-ur-Rahman,2005). Many traditional healing herbs and their parts have been shown to have medicinal value and can be used to prevent, alleviate, or cure several human diseases (Gilani and Atta-ur-Rahman, 2005).

Many drugs commonly used today are of herbal origin. Indeed, about 25 percent of the prescription drugs dispensed in Nigeria contain at least one active ingredient derived from plant material. Some are made from plant extracts; others are synthesized to mimic a natural plant compound. Major pharmaceutical companies conduct extensive research on plant materials gathered from the rain forests and other places for their potential medicinal value, as a result of modern isolation techniques and pharmacological testing procedures, new plant drugs usually find their road into medicines as purified substances(Krishna, 2012).

1.2 Statement of Research Problem

Schizophrenia is a major public concern and it presents a lay concept of insanity. Schizophrenia is a mental disorder characterized by multiple symptoms affecting thought, emotion, perception, volition and general wellbeing of the sufferers (Abdulwakeel *et al.*, 2017). Individuals with schizophrenia are more prone to suicide, depression, anxiety, aggression, substance abuse, cognitive impairment, victimization, poverty and increased medical problems; this makes the disease a serious psychiatric illness among the various nationalities of the world (Addington *et al.*, 2004; Mullen, 2006).

An estimated 2% of people worldwide experience an episode of psychosis in their lifetime, with 80% of these people experiencing the episodes between the ages of 16 and 40 years (Sharifi *et al.,* 2015). In Nigeria, 1.7 million people suffer from schizophrenia (Adegbaju, 2014). An estimated 17,000 people worldwide died from behavior related to, or caused by, schizophrenia (Global Burden of Disease, 2015). The proportion of adults living with psychosis per year in Nigeria was estimated to be between 0.1 and 0.4 per 1000 population (Umukoro, 2016).

After the rise of pharmaceutical industry in the last century and significant progress in the treatment, a period of disappointment comes in accepting the fact that synthetic drugs are not extremely good because they are associated with untoward effects and the overall functional and quality of life outcomes of patients still remain poor after treatment(Mullen, 2006; Magaji *et al.,* 2014) Due to this fact, There is therefore an unmet need in the management of schizophrenia. Thus, there is a critical need to search for more effective and less toxic therapeutic agents to manage psychosis.

1.3 Justification of Study

Schizophrenia is a severe and debilitating mental disorder, afflicting approximately 1% of the world population (Van *et al.,* 2010). Antipsychotic agents are the cornerstone of acute and maintenance treatment of schizophrenia and are effective in the treatment of hallucinations, delusions, and thought disorders, regardless of the cause.In Nigeria, Traditional practitioners use varieties of medicinal plants in the management of psychosis locally such as *Tapinanthus dodoneifolius.*

There is a worldwide 'green revolution (Madhu, 2012) which is reflected in the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs. Furthermore, underlying this upsurge of interest in plants is the fact that many important drugs in use today were derived from plants or from starting molecules of plant origin.Antipsychotic agents in use are less effective on certain symptoms of schizophrenia in addition to serious side effects (Pratt *et al.,* 2012; Abdulwakeel *et al.,* 2017).Herbal medicines form the cornerstone of therapy for management of psychiatric disorders including psychotic symptoms and schizophrenia (Ibrahim *et al.,* 2007; Magaji *et al*., 2008; Ahmed and Azam, 2014).

Majority of the plants used in traditional medicine on central nervous system lack scientific verification. However, only limited efforts have been made to evaluate the potentials of such plants for their use in modern medicine (Yaro *et al.,* 2007).

1.4 Aim and Objectives of the Study

1.4.1 Aim

The aim of this study was to provide pharmacological rationale for the use of *Tapinanthus dodoneifolius* in the management of mental disorder in traditional medicine and the toxicity profile of *Tapinanthus dodoneifolius.*

1.4.2 Objectives

This study was designed to:

i. Establish the class of phytochemical constituents of methanol whole plant extract of *Tapinanthus dodoneifolius.*
ii. Establish the median lethal dose(LD_{50}) and sub chronic toxicity profile of methanol whole plant extract of*Tapinanthus dodoneifolius.*

iii. Determine the antipsychotic effect of methanol whole plant extract of *Tapinanthus dodoneifolius* in mice and rats

1.5 Research Hypothesis

Methanol whole plant extract of *Tapinanthus dodoneifolius* possesses significant antipsychotic activity and it is nontoxic.

CHAPTER TWO

2.0 LITRATURE REVIEW

Psychosisis a severe mental condition in which a sufferer experiences a distortion or loss of contact with reality and clouding of consciousness. It is characterized by depression, delusion, hallucination, anxiety, sleep disturbance, thought disorder, social withdrawal and impaired role functioning (Joel *et al.*,1996). Additionally, a psychotic brain is marked by the insufficient development of cell bodies and the neuronal processes of schizophrenic nerve cells, leading to reductions in the thickness of the cortex (Shuji, 2013). To recognize the importance of mental wellness, 10thOctober every year is marked as World Mental Health Day. This is a day for global mental health education, awareness and advocacy against social stigma.

Psychotic disorders are severe mental disorders of abnormal thinking and perceptions somewhat like losing touch with reality. There are different types of psychotic disorders: schizophrenia, brief reactive psychosis, organic psychosis, delusional disorder, bipolar disorder, psychotic depression and schizoaffective disorder. Schizophrenia is often taken as the psychotic disorder that encompasses all the signs and symptoms known of psychotic disorders (Shuji, 2013).

2.1 Overview of Schizophrenia

The word schizophrenia—which translates roughly as "splitting of the mind" and comes from the Greek roots schizein (σχίζειν, "to split") and phrēn, phren- (φρήν, φρεν-, "mind") was coined by Eugen Bleuler in 1908 and was planned to describe the separation of function between personality, thinking, memory, and perception (Kuhn and Cahn, 2004)

Bleulerinterpretation of schizophrenia led to the claim that he described its main symptoms as four A's: flattened affect, autism, impaired association of ideas, and ambivalence (McNally, 2009). Bleuler also realized that some of his patients improved from the illness rather than deteriorated, and thus proposed the term schizophrenia instead of dementia. Treatment was revolutionized in the mid-1950s with the development and introduction of chlorpromazine(Turner, 2007).

Schizophrenia is a mental disorder which is characterized byfailure to understand reality and unusual social behaviour. Common symptoms include false beliefs, unclear or confused thinking, hearing voices that others do not hear, reduced social engagement and emotional expression, and a lack of motivation. People with schizophrenia often have additional mental health problems such as anxiety, depressive, or substance-use disorders. Symptoms typically come on gradually, begin in young adulthood, and last a long time (Ferri, 2010)

Comment [U1]:

2.2 Epidemiology of Schizophrenia

Schizophrenia affects around 0.3–0.7% of people at some point in their life, or 24 million people worldwide as of 2011. It occurs 1.4 times more frequently in males than females and typically appears earlier in men, the peak ages of onset are 25 years for males and 27 years for females.Onset in childhood is much rarer, as is onset in middle or old age (Cascio*et al.*, 2012). Globally, it has been said that schizophrenia occurs at similar rates, across and within countries (Kirkbride *et al.,* 2007). This variation has been estimated to be fivefold. Schizophrenia causes approximately one percent of worldwide disability adjusted life years and resulted in 20,000 deaths in 2010 (Lozano *et al.,* 2012)

In 2000, the World Health Organization found the percentage of people affected and the number of new cases that develop each year is roughly similar around the world, with age-standardized prevalence per 100,000 ranging from 343 in Africa to 544 in Japan and Oceania for men, and from 378 in Africa to 527 in Southeastern Europe for women and about 1.1% of adults have schizophrenia in the United States.

2.3 Causes of Schizophrenia

Combinations of genetic and environmental factors play a role in the development of schizophrenia. People with a family history of schizophrenia who have a transient psychosis have a 20–40% chance of being diagnosed one year later (Drake and Lewis, 2005)

2.3.1 Genetic factor

Estimates of the heritability of schizophrenia are around 80%, which implies that 80% of the individual differences in risk to schizophrenia are explained by individual differences in genetics. These estimates vary because of the difficulty in separating genetic and environmental influences. The greatest single risk factor for developing schizophrenia is having a first-degree relative with the disease (risk is 6.5%); more than 40% of monozygotic twins of those with schizophrenia are also affected. If one parent is affected the risk is about 13% and if both are affected the risk is nearly 50% (Combs *et al.,* 2011)

Many genes are known to be involved in schizophrenia, each of small effect and unknown transmission and expression (Schork *et al.,* 2016). The summation of these effect sizes into a polygenic risk score can explain at least 7% of the variability in liability for schizophrenia (Lowther *et al.*, 2017). Around 5% of cases of schizophrenia are understood to be at least partially attributable to rare copy number variants (CNVs), including 22q11, 1q21 and

16p11. These rare CNVs increase the risk of an individual developing the disorder by as much as 20-fold, and are frequently co morbid with autism and intellectual disabilities. There is a genetic relation between the common variants which cause schizophrenia and bipolar disorder, an inverse genetic correlation with intelligence and no genetic correlation with immune disorders (Opler *et al.*, 2005; Kendler, 2016)

2.3.2 Environmental factor

Environmental factors associated with the development of schizophrenia include the living environment, drug use, and prenatal stressors.

Maternal stress has been associated with an increased risk of schizophrenia, possibly in association with reelin. Maternal Stress has been observed to lead to hypermethylation and therefore under-expression of reelin, which in animal models leads to reduction in GABAergic neurons, a common finding in schizophrenia. Maternal nutritional deficiencies, such as those observed during a famine, as well as maternal obesity have also been identified as possible risk factors for schizophrenia. Both maternal stress and infection have been demonstrated to alter fetal neurodevelopment through pro-inflammatory proteins such as IL-8 and TNF (Brown, 2011)

Parenting style seems to have no major effect, although people with supportive parents do better than those with critical or hostile parents. Childhood trauma, death of a parent, and being bullied or abused increase the risk of psychosis. Living in an urban environment during childhood or as an adult has consistently been found to increase the risk of schizophrenia by a factor of two, even after taking into account drug use, ethnic group, and size of social group. Other factors that play an important role include social isolation and

immigration related to social adversity, racial discrimination, family dysfunction, unemployment, and poor housing conditions (Picchioni and Murray, 2007)

It has been hypothesized that in some people, development of schizophrenia is related to intestinal tract dysfunction such as seen with non-celiac gluten sensitivity or abnormalities in the intestinal flora. A subgroup of persons with schizophrenia present an immune response to gluten different from that found in people with celiac, with elevated levels of certain serum biomarkers of gluten sensitivity such as anti-gliadin IgG or anti-gliadin IgA antibodies (Lachance and McKenzie, 2014)

2.3.3 Substance use

About half of those with schizophrenia use drugs or alcohol excessively (Gregg *et al.,* 2007) Amphetamine, cocaine, and to a lesser extent alcohol, can result in a transient stimulant psychosis or alcohol-related psychosis that presents very similarly to schizophrenia. Although it is not generally believed to be a cause of the illness, people with schizophrenia use nicotin eat much higher rates than the general population (Sagud *et al.,* 2009) Alcohol abuse can occasionally cause the development of a chronic, substance-induced psychotic disorder via a kindling mechanism. Alcohol use is not associated with an earlier onset of psychosis (Large *et al.,* 2011)

Cannabis can be a contributory factor in schizophrenia, potentially causing the disease in those who are already at risk. The increased risk may require the presence of certain genes within an individual or may be related to preexisting psychopathology. Early exposure is strongly associated with an increased risk. The size of the increased risk is not clear, but appears to be in the range of two to three times greater for psychosis. Higher dosage and

greater frequency of use are indicators of increased risk of chronic psychoses (Niesink and VanLaar 2013)

Other drugs may be used only as coping mechanisms by individuals who have schizophrenia, to deal with depression, anxiety, boredom, and loneliness (Leweke and Koethe, 2008)

2.3.4 Developmental factors

Factors such as hypoxia and infection, or stress and malnutrition in the mother during foetal development, may result in a slight increase in the risk of schizophrenia later in life. People diagnosed with schizophrenia are more likely to have been born in winter or spring (at least in the northern hemisphere), which may be a result of increased rates of viral exposures in utero. The increased risk is about five to eight percent. Other infections during pregnancy or around the time of birth that may increase the risk include *Toxoplasma gondi* and Chlamydia(Arias *et al.,* 2012)

2.4 Hypothesis of Schizophrenia

A number of attempts have been made to explain the link between altered brain function and schizophrenia. One of the most common is the Dopamine hypothesis, which attributes psychosis to the mind's faulty interpretation of the misfiring of Dopaminergic neurons(Kuhn and Cahn, 2004)

2.4.1 Psychological

Many psychological mechanisms have been implicated in the development and maintenance of schizophrenia. Cognitive biases have been identified in those with the diagnosis or those at risk, especially when under stress or in confusing situations. Some cognitive features may

reflect global neurocognitive deficits such as memory loss, while others may be related to particular issues and experiences (Bentall *et al.,* 2007).

Despite a demonstrated appearance of blunted affect, recent findings indicate that many individuals diagnosed with schizophrenia are emotionally responsive, particularly to stressful or negative stimuli, and that such sensitivity may cause vulnerability to symptoms or to the disorder. Some evidence suggests that the content of delusional beliefs and psychotic experiences can reflect emotional causes of the disorder, and that how a person interprets such experiences can influence symptomatology (Smith *et al.,* 2010) The use of "safety behaviors" (acts such as gestures or the use of words in specific contexts) to avoid or neutralize imagined threats may actually contribute to the chronicity of delusions. Further evidence for the role of psychological mechanisms comes from the effects of psychotherapies on symptoms of schizophrenia. (Kuiper*set al.,* 2006)

2.4.2 Neurological

Schizophrenia is associated with subtle differences in brain structures, found in forty to fifty percent of cases, and in brain chemistry during acute psychotic states (Van and Kapur, 2009) Studies using neuropsychological tests and brain imaging technologies such as fMRI and PET to examine functional differences in brain activity have shown that differences seem to occur most commonly in the frontal lobes, hippocampus and temporal lobes (Kircher and Thienel, 2006) Reductions in brain volume are most pronounced in grey matter structures, and correlate with duration of illness, although white matter abnormalities have also been found. A progressive increase in ventricular volume as well as a progressive reduction in grey matter in the frontal, parietal, and temporal lobes has also been observed. These differences have been linked to the neurocognitive deficits often associated with

schizophrenia. Because neural circuits are altered, it has alternatively been suggested that schizophrenia could be thought of as a neurodevelopmental disorder with psychosis occurring as a possibly preventable late stage. There has been debate on whether treatment with antipsychotics can itself cause reduction of brain volume (Kuhn and Cahn, 2004)

Particular attention has been paid to the function of dopamine in the mesolimbic pathway of the brain. This focus largely resulted from the accidental finding that phenothiazine drugs, which block dopamine function, could reduce psychotic symptoms. It is also supported by the fact that amphetamines, which trigger the release of dopamine, may exacerbate the psychotic symptoms in schizophrenia (Laruelle *et al.,* 1996).

The influential dopamine hypothesis of schizophrenia proposed that excessive activation of D_2 receptors was the cause of (the positive symptoms of) schizophrenia. Although postulated for about 20 years based on the D_2 blockade effect common to all antipsychotics, it was not until the mid-1990s that PET and SPECT imaging studies provided supporting evidence. While dopamine D2/D3 receptors are elevated in schizophrenia, the effect size is small, and only evident in medication naive schizophrenics on the other hand, presynaptic dopamine metabolism and released is elevated despite no difference in dopamine transporter. The altered synthesis of dopamine in the nigrostriatal system have been confirmed in several human studies. Hypoactivity of dopamine D1 receptor activation in the prefrontal cortex has also been observed (Salavati *et al.,* 2015) The hyperactivity of D2 receptor stimulation and relative hypoactivity of D1 receptor stimulation is thought to contribute to cognitive dysfunction by disrupting signal to noise ratio in cortical microcircuits (Winterer and Weinberger, 2004) The dopamine hypothesis is now thought to be simplistic, partly because newer antipsychotic medication (atypical

antipsychotic medication) can be just as effective as older medication (typical antipsychotic medication), but also affects serotonin function and may have slightly less of a dopamine blocking effect (Jones and Pilowsky, 2002).

Interest has also focused on the neurotransmitter glutamate and the reduced function of the NMDA glutamate receptor in schizophrenia, largely because of the abnormally low levels of glutamate receptors found in the postmortem brains of those diagnosed with schizophrenia, and the discovery that glutamate-blocking drugs such as phencyclidine and ketamine can mimic the symptoms and cognitive problems associated with the condition. Reduced glutamate function is linked to poor performance on tests requiring frontal lobe and hippocampal function, and glutamate can affect dopamine function, both of which have been implicated in schizophrenia; this has suggested an important mediating (and possibly causal) role of glutamate pathways in the condition. But positive symptoms fail to respond to glutamatergic medication. Closely related to evidence of glutamate dysfunction in schizophrenia is the observed changes GABAergic transmission. Post-Mortem studies demonstrate decreased expression of GAD67, GAT-1 and $GABA_A$ receptor subunits in the prefrontal cortex, although this appears to be restricted to a certain subsets of parvalbumin containing GABAergic neurons. While in vivo imaging of GABAergic signaling appears to be moderately reduced, this may be dependent upon treatment and disease stage (Walton*et al.,* 2017).

2.5 Signs and Symptoms Schizophrenia

Schizophrenia is often described in terms of positive and negative (or deficit) symptoms.

2.5.1 Positive symptoms; are those that most individuals do not normally experience, but are present in people with schizophrenia. They can include delusions, disordered thoughts and speech, and tactile, auditory, visual, olfactory and gustatory hallucinations, typically regarded as manifestations of psychosis. Hallucinations are also typically related to the content of the delusional theme. Positive symptoms generally respond well to medication (Smith *et al.,* 2010).

2.5.2 Negative symptoms; are deficits of normal emotional responses or of other thought processes, and are less responsive to medication. They commonly include flat expressions or little emotion, poverty of speech, inability to experience pleasure, lack of desire to form relationships, and lack of motivation. Negative symptoms appear to contribute more to poor quality of life, functional ability, and the burden on others than positive symptoms do. People with greater negative symptoms often have a history of poor adjustment before the onset of illness, and response to medication is often limited (Smith *et al.,* 2010).

2.6 Diagnosis of schizophrenia

Schizophrenia is diagnosed based on criteria in either the American Psychiatric Association's (APA) fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM 5), or the World Health Organization's International Statistical Classification of Diseases and Related Health Problems (ICD-10). These criteria use the self-reported experiences of the person and reported abnormalities in behavior, followed by a clinical assessment by a mental health professional. Symptoms associated with schizophrenia occur along a continuum in the population and must reach a certain severity and level of impairment, before a diagnosis is made (Picchion and Murry, 2007)

2.7 Subtypes of Schizophrenia

With the publication of DSM-5, the APA removed all sub-classifications of schizophrenia. The five sub-classifications included in DSM-IV-TR were:

2.7.1 Paranoid type: Delusions or auditory hallucinations are present, but thought disorder, disorganized behavior, or affective flattening are not. Delusions are persecutory and/or grandiose, but in addition to these, other themes such as jealousy, religiosity, or somatization may also be present (DSM code 295.3/ICD code F20.0).

2.7.2 Disorganized type: Named hebephrenic schizophrenia in the ICD. Where thought disorder and flat affect are present together (DSM code 295.1/ICD code F20.1).

2.7.3 Catatonic type: The subject may be almost immobile or exhibit agitated, purposeless movement. Symptoms can include catatonic stupor and waxy flexibility (DSM code 295.2/ICD code F20.2).

2.7.4 Undifferentiated type: Psychotic symptoms are present but the criteria for paranoid, disorganized, or catatonic types have not been met (DSM code 295.9/ICD code F20.3).

2.7.5 Residual type: Where positive symptoms are present at a low intensity only (DSM code 295.6/ICD code F20.5).

2.8OVERVIEW OF ANTIPSYCHOTIC DRUGS

2.8.1 Classification:Antipsychotic drugs are broadly divided into typical and atypical antipsychotics;

The *Typical antipsychotics* are the older agents, are also referred to as first generation antipsychotics, classical antipsychotics, dopamine receptor antagonists or neuroleptics. they can further be divided into different classes based on certain criteria- for example, based on their ability to block dopamine receptors, they can be divided into low potency typical antipsychotics (chlorpromazine and thioridazine) and high potency typical antipsychotics (such as haloperidol, fluphenazine and flupenthixol) (Leucht*et al.,* 2012)

The *Atypical antipsychotics* are the newer agents, also referred to as second generation drugs or limbic-specific Antipsychotics (Leucht*et al.,* 2012).

Drug groups	**examples**
Typical Antipsychotics	
Phenothiazine	
Aliphatic	Chlorpromazine, Promazine
Piperazine	Fluphenazine, Trifluperazine,Perphenazine
Piperidine	Thioridazine, Pericyazine
Butyrophenone	Haloperidol, Benperidol

Thioxanthine	Flupentixol,Zuclophenthixol

Atypical Antipsychotics

Dibenoxazepine	Tricyclics Cloxapine, Loxapine
Thienobenzodiazepines	Olanzepine, Quetiapine,Zoetepine
Serotonin-Dopamine Antagonists	Risperidone, Ziprasidone

2.8.2 Mechanism of action

A better understanding of Antipsychotic actions based on the knowledge of central dopamine pathways; the central dopamine system is composed of four pathways, each with its own specialized function;

Mesolimbic dopamine pathway: Projects from the midbrain (ventral tegmental area,) to the limbic system (nucleus of accumbens). It is believed to be involved in delusions and hallucinations of psychosis and many other behaviors such as pleasurable sensations and the powerful euphoria of drugs abuse. Blockage of dopamine receptors in this pathway results in the reduction of positive symptoms of schizophrenia. This blockade is desirable in schizophrenia treatment (Meltzer, 2013)

Mesocortical dopamine pathway: Projects from the midbrain (ventral tegmental area,) to the cortex. It is believed to be responsible for higher-order thinking and executive functions. Blockade of dopamine receptors in this pathway may be responsible for negative symptoms (Meltzer, 2013)

Nigrostriatal dopamine pathway: Projects from the substantianigra to the basal ganglia. It is believe to control movements. Blockade of dopamine receptors in this pathway causes Extra Pyramidal Symptoms (EPS) (Meltzer, 2013)

Tuberoinfundibular dopamine pathway: Projects from the hypothalamus to the anterior pituitary. It is believe to control prolactin secretion. Blockade of dopamine receptors in this pathway results in hyperprolactinemia (Meltzer, 2013).

2.8.3 Side effects or extra pyramidal symptoms (EPS)

Extra Pyramidal Symptoms (EPS) is a broad term that describes several types of acute and chronic movement disorders that occurs as a result of central dopamine receptor blockage, usually by antipsychotics. These set of symptoms can be classified into four groups;

Acute Dystonia; Characterized by sustained muscle contraction (usually in the head and neck region) which can occur within the first few hours or days after initiation or dose increase of antipsychotics, especially if given in high doses or by injection. Thus, it has the earliest onset of all (EPS) symptoms. It present as grimacing and facial distortion, neck twisting and labored breathing. Risk factors include younger age, male gender, and high dosage of high potency typical antipsychotics and previous history of dystonia. Treatment;for immediate treatment, parental anticholinergics (benztropine, diphenhydramine and procyclidine) or benzodiazepine (lorazepam) can be used. To prevent another reaction, oral Anticholinergics can be used, usually for two weeks (Ellenbroek, 2012).

Akathisia; Characterized by marked restlessness and anxiety that can occur with days to weeks of initiating antipsychotics therapy. It is often involved at the lower extremities. Akathisia can complicate schizophrenia treatment because if allowed to persist, it can produce dysphoria and possibly aggressive or suicidal behavior. It is difficult to distinguish from psychomotor agitation and worsening of psychosis. Thus, care must be taken not to misdiagnose it because increase dose of antipsychotics can worsen this adverse effect. It can be extremely distressing to patients and therefore, can have strong influence on compliances. Treatmentinvolved is usually less responsive than dystonia and AIP(Antipsychotic induced parkinsonism). Reduction in the antipsychotics dose (if appropriate), Addition of an adjunct drugs such as Anticholinergics (benzhexol), Beta blocker (propranolol), Benzodiazepine (diazepam), or switch to Atypical Antipsychotics (Ellenbroek, 2012).

Parkinsonism: This can be referred to as Antipsychotic-induced Parkinsonism. It can occur at any time but usually develop within four weeks after initiating antipsychotic therapy or dose increase. It usually present with tremor, rigidity of the extremities, neck or trunk, decreased arm swinging, masklike face, decreased eye blinking, drooling, and excessive salivation. Treatment involves; reduction of dose in the antipsychotics, addition of an antiparkinsonian agent such as anticholinergic(benzhexol), GABAminergic(diazepam), Dopaminergic (amantadine), Noradrenergic (propranolol) (Ellenbroek, 2012).

Tardive dyskinesia (TD): This is a syndrome that can develop months or years after initiating therapy. It consist of abnormal involuntary movements of the face, tongue, lips, and limbs. The exact cause of TD is not known but there are believes that Dopaminergic hypersensitivity and disturbed balance between dopamine and cholinergic systems are better

explanations. Treatment involves recommendation switch to atypical antipsychotics (Ellenbroek, 2012)

Other side effects of Typical Antipsychotics with low potency and some atypical antipsychotics include; sedation, Anticholinergic effects(constipation, urinary retention, blurred vision, dry eyes, mouth and throat.) seizures, sexual dysfunction, cardiovascular effects(tachycardia, orthostatic or postural hypertension), neuroleptic malignant syndrome (more common with typical antipsychotics) (Picchioni and Murry, 2007)

2.9 Prognosis

Schizophrenia has great human and economic costs. It results in a decreased life expectancy by 10–25 years, due to its association with obesity, poor diet, sedentary lifestyles, and smoking, with an increased rate of suicide playing a lesser role. Antipsychotic medications may also increase the risk. These differences in life expectancy increased between the 1970s and 1990s (Saha *et al.*, 2007; Laursen *et al.*, 2012)

Schizophrenia is a major cause of disability, with active psychosis ranked as the third-most-disabling condition after quadriplegia and dementia and ahead of paraplegia and blindness (Ustün*et al.,* 1999). Approximately three quarter of people with schizophrenia have ongoing disability with relapses and 16.7 million people globally are deemed to have moderate or severe disability from the condition. Some people do recover completely and others function well in society. Most people with schizophrenia live independently with community support. About 85% are unemployed (Owen *et al.*, 2016) some evidence suggests that paranoid schizophrenia may have a better prospect than other types of schizophrenia for independent living and occupational functioning (Menezes *et al.*, 2006) In people with a first episode of

psychosis a good long-term outcome occurs in 42%, an intermediate outcome in 35% and a poor outcome in 27%. Outcomes for schizophrenia appear better in the developing than the developed world. These conclusions, however, have been questioned (Burns, 2009)

There is a higher than average suicide rate associated with schizophrenia. This has been cited at 10%, but a more recent analysis revises the estimate to 4.9%, most often occurring in the period following onset or first hospital admission. Several times more (20 to 40%) attempt suicide at least once. There are a variety of risk factors, including male gender, depression, and a high intelligence quotient (Carlborg *et al.,* 2010)

Schizophrenia and smoking have shown a strong association in studies worldwide. Use of cigarettes is especially high in individuals diagnosed with schizophrenia, with estimates ranging from 80 to 90% being regular smokers, as compared to 20% of the general population. Those who smoke tend to smoke heavily, and additionally smoke cigarettes with high nicotine content. Among people with schizophrenia use of cannabis is also common (Keltner and Grant, 2006; Gregg *et al.,* 2007)

2.10 Pharmacological Model of Schizophrenia

Three main neurochemical hypotheses of schizophrenia have been developed, according to the effects of drugs that interfere with the neurotransmitters dopamine, glutamate, and serotonin. Drugs interfering with these systems induce certain behavioral changes in laboratory animals that can be reversed by antipsychotics (Pedro *et al.,* 2013).

Dopaminergic hypotheses of schizophrenia

The dopamine theory was proposed by Carlson-awarded a Nobel Prize in 2000-on the basis of indirect pharmacological evidence in humans and experimental animals. Dopamine is a

monoamine neurotransmitter which is involved in other diseases, such as Parkinson's disease. Animal models were first produced for schizophrenia by altering the dopaminergic system using drugs. Persistent treatment of rodents with Amphetamine, apomorphine, bromocriptine which act as potent agonist a D2-receptor. Activation of D2-receptor produces positive symptoms of schizophrenia (due to stimulation of mesolimbic pathway) and negative symptoms schizophrenia (due to stimulation of mesocortical pathway) in animals. Administration of antipsychotics like; clozapine and haloperidol reverse the effects of amphetamine induced schizophrenia in animals. Several pieces of evidence corroborate this hypothesis. Recently, studies using positron emission tomography imaging indicate that there is an increase in dopamine synthesis in drug-naïve schizophrenic patients as compared with age-matched controls (Brunelin *et al.,* 2010) It was also demonstrated that amphetamine induces a greater increase in dopamine release in drug naive patients as compared with controls (Breier *et al.,* 1997) Together, these data reinforce the role of dopamine in schizophrenia. In this way, some important experimental models used to study schizophrenia consist of quantification of behaviors in response to the administration of dopaminergic drugs, such as amphetamine, which facilitates dopamine release; cocaine, which inhibits dopamine reuptake; or apomorphine, which activates the dopamine D2 receptor directly (Mouri *et al.,* 2013)

Glutamate hypothesis of schizophrenia

Glutamate is the most abundant excitatory neurotransmitter in vertebrate nervous systems. Evidence for the involvement of glutamate in schizophrenia includes analogous symptoms which are produced by glutamate NMDA receptor antagonists such as phencyclidine (PCP) and ketamine.PCP is a non-competitive NMDA receptor antagonist which produces

hallucinations and delusions in normal subjects. In rat models, disturbed cognition, deficits in social interaction, locomotor anomalies, and prepulse inhibition deficits are seen on acute administration of PCP. The effect of PCP or ketamine induced schizophrenia can be reversed by administration of antipsychotics like clozapine, haloperidol. (Pratt *et al.*, 2012; Coyle *et al.*, 2012)

Serotonin (5-ht) hypothesis of schizophrenia

Serotonin induce schizophrenia is based on the attribution of the psychotomimetic effects of indolamine derivative such as lysergic acid diethylamide (LSD, which is structurally related to 5-HT) and Phenethylamines (e.g mescaline) in the brain antagonize 5-HT2A receptors(Meltzer and Massey, 2011). In rodents, acute or chronic treatment with these drugs induces behavioral abnormalities such as scratching, forepaw treading, head twitches, and lower lip retraction. Some of these effects may depend on functional interplay between dopamine and serotonin pathways. Indeed, some studies report that there is a decrease in the density of 5-HT2A receptors in the prefrontal cortex of patients, while there was a significant increase in the density of dopamine D2 receptors in the caudate nucleus, suggesting that dysfunction in serotoninergic activity could contribute to the alteration of dopaminergic function seen in schizophrenia. The discovery that the effects of some antipsychotics may be due, at least in part, to their binding to various5-HT receptors, especially 5-HT2A and 5-HT1A, also provides evidence that serotonin may play an important role in schizophrenia. In this context, the serotoninergic models of schizophrenia are obtained by injection of direct agonists, such as the hallucinogen DOI (a substance that resembles LSD) or serotonin-releasing agents, such as MDMA (Meltzer and Massey, 2011)

The major limitations with this hypothesis were;

The recognition that the primary effect of LSD was to produce visual hallucinations, which are relatively rare in schizophrenia, not auditory hallucinations, which are the most common perceptual disturbance in schizophrenia. LSD is a full or partial agonist rather than an antagonist at many 5-HT receptors.

It has been shown that atypical antipsychotic e.g clozapine, olanzapine have higher affinity for serotonin receptor, thus, reversing the effect of LSD induce schizophrenia (Rauser *et al.*, 2011).

Gabanergic hypothesis of schizophrenia

Aminobutyric acid (GABA) is a major inhibitory neurotransmitter. The GABAergic system may be involved in schizophrenia due to its interactions with the dopaminergic system.Picrotoxin, an antagonist for the GABA-A receptor, produces prepulse inhibition of startle in rats. Haloperidol and other antipsychotics, reduces this effect.

2.11 Tests that predict the efficacy of antipsychotic drugs

The detection of antipsychotic activity in laboratory animals consists on evaluating whether a drug is able to prevent or reverse certain behavioral alterations, which can be induced by the pharmacological agents. Particularly relevant are the conditioned avoidance response (CAR), stereotypies, hyperlocomotion, and disruption in the prepulse inhibition of the startle reflex (PPI) (Pedro *et al.*, 2013)

Conditioned Avoidance Response

The CAR does not require further pharmacological intervention to induce a behavioral abnormality (i.e., it is not a pharmacological model). This is one of the oldest and most classical tests predictive of the therapeutic effects of antipsychotics agents. In the CAR,

animals are trained to avoid the occurrence of an aversive stimulation, usually an electric shock, by making a specific behavioral response in a shuttle box, such as moving to the other side of the box.

Antipsychotics block the CAR in doses that do not interfere with escape after stimulus onset and correlate well with clinically used doses. Interestingly, the percentage of striatal D2 receptor occupation required to inhibit the CAR is around 70%, similar to the threshold required for the therapeutic effect of antipsychotics in humans. In addition, this paradigm does not tend to yield false-positive results with sedative drugs (e.g., benzodiazepines), since these normally impair both the avoidance and the escape responses. Regarding its predictive validity, this model is reliable for identifying new drugs, in addition to being simple, quick, and low-cost. Its face validity, however, is low (Wadenberg, 2010)

Stereotypies

Stereotypy, a distinct feature of schizophrenia, is characterized by repetitive, unvarying, and functionless behavior. More recently, stereotypy has been defined as comprising strictly repetitive motor actions, thus distinguishing it from perseveration, a cognitive behavior. It can be induced in rats and manifests as licking of the paws as well as smelling and biting the cage bars, which are part of the normal behavior of this species.

These responses can be induced by direct (apomorphine) and indirect (amphetamine) dopaminergic agonists. They seem to result from the stimulation of D_2 receptors located in the dorsal striatum rather than in the nucleus accumbens, the supposed site for the therapeutic activity of antipsychotic drugs.

Various typical and atypical antipsychotics inhibit apomorphine- and amphetamine-induced stereotypies, although clozapine has been found less effective against this alteration,

reflecting its supposed preferential action on D_2 receptors in the limbic system, as compared to the dorsal striatum. NMDA receptor antagonists also produce stereotypies, and the disturbance produced by these compounds, whether acutely or chronically administered, can also be prevented by treatment with antipsychotic drugs (Bondi *et al.*, 2003) the advantage of this test is that stereotypies can be easily quantified by a trained observer, at minimal cost and yielding rapid results. The reversion of this behavior by antipsychotic drugs demonstrates its predictive validity. Nonetheless, the fact that this behavior seems to depend on dopaminergic action in the dorsal striatum rather in the nucleus accumbens (the supposed site for antipsychotic drug action) indicates that its construct validity is limited.

Hyperlocomotion

The increase in locomotion induced by certain drugs in experimental animals may be representative of the positive symptoms of schizophrenia. This response results from increased dopaminergic activity in the mesolimbic pathway projecting to the nucleus accumbens. It can be evoked by administration of direct and indirect dopaminergic agonists or NMDA receptor antagonists. As for its predictive validity, hyperlocomotion is efficaciously prevented by treatment with both typical and atypical antipsychotics. Nonetheless, compounds that induce sedative effects per se may yield false positives. Thus, an obligatory control is testing the effects of candidate drugs on baseline spontaneous locomotion, so as to ensure that the reversal of hyperlocomotion is not secondary to a sedative property of the drug. Another problem is the lack of standardization for test duration, light intensity in the room, and size of the arena, among other parameters. Despite

these drawbacks, this test has a low cost and good reproducibility. In addition, this response is easily assessed, making it appropriate for an initial screening for new candidate antipsychotic drugs in rats and mice (Peleg-Raibstein*et al.*, 2012)

Locomotion can be quantified inside a round or square arena or open field, in which automated tracking systems enable rapid and reliable analysis of drug effects. This can be achieved by recording a normal arena with a camera coupled to computer software or by an arena equipped with light beams that quantify both horizontal and vertical movement of the animals.

Disruption of the prepulse inhibition of the startle response

The PPI seeks to explore the information processing deficits that typically occur in patients with schizophrenia. Normally, loud unexpected stimuli elicit a typical response termed the startle reflex. However, if this sudden, intense startling stimulus (pulse) is preceded by a weaker, nonstartling sensory stimulus (prepulse), the startle response is inhibited (hence, prepulse inhibition). Patients suffering from schizophrenia have a deficit in PPI, meaning that they exhibit the startle response even when the pulse is preceded by the weak stimulus. Treatment with direct or indirect dopamine agonists mimics information processing deficits characteristic of schizophrenia, thus impairing PPI. This is reversed by several antipsychotics, such as clozapine, haloperidol, chlorpromazine, risperidone, and quetiapine. Serotonin drugs such as DOI or MDMA are also able to induce a PPI deficit. Finally, another pharmacological mechanism to disrupt PPI is the antagonism of NMDA receptors. This model, contrary to the others mentioned above, may be able to distinguish the effects promoted by atypical vs. typical antipsychotics. This profile is similar to that observed after administration of NMDA receptor antagonists to humans (Geyer and Ellenbroek, 2003) in

humans, the startle reflex can be measured as a contraction of the skeletal and facial muscles, such as an eye-blink reflex. This phenomenon occurs consistently across species and can be assessed in laboratory mice and rats using similar stimuli (tone presentations) and measuring the startle by placing the animal over a platform that detects its movement. This test has been receiving wider attention due to its face validity. Nonetheless, it requires a more expensive apparatus (the startle box) and one must control for drug effects on the startle response itself. A specific antipsychotic effect occurs when a drug restores PPI (in the prepulse-pulse sequence) without interfering with the response to the pulse alone (which would be indicative of a motorimpairing effect) (Fendt and Yeomans,2001)

Tests relevant to the negative and cognitive symptoms of schizophrenia

Some behavioral tests, though not specifically related to schizophrenia and antipsychotic activity, may be useful in predicting efficacy against negative symptoms. Most of them consist of detecting the reversal of certain deficits induced by sub-anesthetic doses of NMDA antagonists. One example is the social interaction test. Social interaction in animals are reduced after treatment with ketamine, phencyclidine, and dizocilpine, and restored by antipsychotics. Interesting, the data have been more consistent with atypical antipsychotics. Nonetheless, this activity is not specific for this class of drugs, since anxiolytic compounds also increase social interaction in rats. In any case, this test is relevant, since current antipsychotic drugs have very limited efficacy against negative symptoms. Likewise, cognitive impairment, a common symptom of schizophrenia, is not improved by currently used medicines. Thus, there is an urgent need for drugs that are able to improve learning and memory deficits in this syndrome. Memory deficits can be induced by several protocols and tested in the object recognition test and the Morris water maze. Again, these tests are not

specific for antipsychotic drugs, which do not consistently reverse memory deficits (Peleg-Raibstein *et al.*, 2012)

2.12 Tests that predict the side effects of antipsychotic drugs

The short-term side effects of first-generation antipsychotic drugs include parkinsonian syndrome, dystonia and akathisia, whereas chronic treatment leads to tardive dyskinesia, which comprises abnormal, excessive, and involuntary movements. Despite a large number of investigations, the mechanisms through which these effects occur remain to be elucidated. These include dopaminergic supersensitivity, excitotoxicity, free radical formation, and a decrease in dopamine transporter density. To detect the liability of compounds to promote such side effects, as well as to understand their mechanisms, some simple behavioral tests can be reliably used (Casey, 2000)

The catalepsy test

Catalepsy in laboratory animals is defined as a failure to correct an externally imposed posture. The test, which is widely used, consists simply of measuring latency for the animal to remove itself from an unusual and uncomfortable posture. The most commonly used assessment is the bar test, in which a mouse or rat is placed with its hindpaws on a bench and its forepaws on a bar elevated a few centimeters. The latency for the animal to move both forepaws from the bar, or to climb it, is then measured. The cutoff time is usually 5 minutes. In another variant, the wire grid test, the animal is positioned on a wire grid at an angle of 50 degrees to the surface. The forelimbs are spread and the time the animal remains in this position is measured. The catalepsy test is frequently used in drug screening to

evaluate the liability of potential antipsychotics to induce extrapyramidal side effects. Generally, the doses required to induce catalepsy occupy approximately 80% of D_2 receptors in the striatum, being higher than those efficacious in models predictive of antipsychotic efficacy, which requires around 65-70% occupation. Depending on the drug, the dose difference required to induce catalepsy and antipsychotic-like effects ranges from very small, as is the case for haloperidol, to high, as for clozapine, which is in line with the clinical profile of these drugs. Indeed, the doses required for antipsychotic drugs to induce catalepsy in animal correlates well with those that induce extrapyramidal side effects in humans. Thus, this test has a very high predictive validity; it is cheap, simple, reproducible, and easy to perform. Factors such as the height of the bar as well as the size of the animal must be well documented (Hoffman and Donovan, 1998)

The vacuous chewing movements test

One of the most concerning side effects of antipsychotics is tardive dyskinesia, which is clinically relevant due to its high prevalence, its impact on quality of life, and the fact that it persists even after treatment discontinuation. The most widely used and accepted test for studying this side effect is carried out in rats, in which the main parameter evaluated is the presence of orofacial dyskinesias, which manifest as vacuous chewing movements (VCMs). (Woods *et al.*, 2010) The test is conducted in rats because mice are smaller and have rapid movements that hinder visualization and quantification of orofacial movements. The validity of the VCMs is based on their similarity with tardive dyskinesia in humans, since the symptoms persist chronically, can fluctuate, and continue after prolonged drug withdrawal. Finally, not all animals exposed to long-term antipsychotic treatment develop VCMs; their

incidence is higher with advancing age and symptoms is exacerbated by stress, which is also consistent with clinical observations in humans (Crowley *et al.*, 2012)

Usually, animals are treated with slow-releasing preparations of antipsychotic drugs, haloperidol and fluphenazine decanoates being most commonly used, at a dose of 1 mg/day during periods ranging from 4 to 36 weeks. For the experimental evaluation, the animals are placed individually in cages withmirrors under the floor, to facilitate visualization of the mouth. After a period of adaptation, the number of VCMs is counted. A VCM is defined as single mouth opening not directed towards physical material. Total duration of facial tremors and the number of tongue protrusions are also evaluated. The appearance of VCMs usually occurs after 3-4 weeks of exposure to the drug (short-term VCMs). However, while long-term VCMs reflect the development of tardive dyskinesia, the short-term VCMs reflect the acute extrapyramidal side effects, and their use to evaluate tardive dyskinesia is controversial (Burger *et al.*, 2005)

2.13 The plant;*Tapinanthus dodoneifolius*

Taxonomic nomenclature

Plant Name: *Tapinanthus dodoneifolius* (D.C) Danser

Kingdom: Plantae

Clade: Angiosperms

Clade: Eudicots

Order: Santalales

Family: Loranthaceae

Genus: Tapinanthus

Species: dodoneifolius

Common Names: English: African Mistletoe; Hausa: Kauchi Dorowa; Yoruba: Afomu Igba; Igbo: Elozie; Nupe: Etu-Ionchi (Deeni and Sadiq, 2002).

2.13.1 Description, origin and geographical distribution

This plant a species of African mistletoe which is bushy, hemi-parasitic in nature, which grows on a wide range of trees such as *Parkia biglobosa, Tamarindus indica, Azadirachta indica*, Kola, citrus, acacia, orange and many other trees as a host plants. It is found in North/Central Namibia, West Africa, North America and Europe. It is a green shrub, the leaves are ovate, round at the apex about 7cm long and 3cm broad with irregular pinna arranged lateral nerves and small purple flowers with white sticky barriers which are considered poisonous (Baso and Mudi, 2017). It grows on the branches, roots and twigs of host plant.

Plate 1:The plant, *Tapinanthus dodoneifolius* DC Danser in its natural habitat(Uthman *et al*., 2015)

2.13.2 Ethnomedicinal uses

It twigs and leaves are used in the treatment of various ailments in different areas around the world. It is used in Northern Nigeria in the treatment of stomach ache, diarrhoea, dysentery, wound and cancer (Deeni and Sadiq, 2002).

In Burkina Faso it is used for the treatment of cardiovascular and respiratory diseases (Oudraogo*et al.,* 2005).The leaves and young twigs of the plant are used to treat malaria, diabetes, hypertension and sterility in cow (Efuntoye *et al.,* 2010). It is also reported to be used in the treatment of diabetes, blood pressure, asthma, epilepsy and cancer (Ekhaise *et al.,* 2010)

In ayurvedic medicine, *Tapinanthus dodoneifolius* is used for the treatment of various diseases such as sciatica, chronic fever, rheumatism, internal worm infections, asthma, inflammations, dyspepsia, dermatitis, bronchitis, cough, constipation, grayness of hair and baldness(Spencer, 2008).

2.13.3 Previous pharmacological studies on *Tapinanthus dodoneifolius*

Previous studies on *Tapinanthus dodoneifolius* showed that it possesses pharmacological activities, these include;

Anticonvulsant activity:

The extract methanol leaf extract of *Tapinanthus dodoneifolius* dc danser, dose dependently protected the rats against mortality caused by pentylenetetrazole induced convulsion (Uthman *et al.,* 2015)

Cardiovascular activity:

Aqueous extract of *Tapinanthus dodoneifolius* was investigated for cardiovascular activities on rat aorta and heart. It was observed that the extract did not affect heart rate but significantly enhanced heart contraction force and relaxation capacity (Oudraogo*et al.*, 2005).

Antimicrobial activity:

Methanol extract of *Tapinanthus* grown on *Phyllanthus muellerianus* showed activity against *Escherichia coli* and *Staphylococcusaureus* while chloroform extract of *Tapinanthus* grown on *Parkia biglobosa* and *Citrus aurantifolia* respectively, showed activityagainst *Escherichia coli* and *Staphylococcus aureus* (Efuntoye *et al.,* 2010).Methanol extractsobtained from *Tapinanthus* inhibit growth of *bacillus* spp., *proteus* spp. and *pseudomonas* spp., a bacterial spp. known to be associatedwith gastrointestinal tract and wound infection(Deeni and Sadiq, 2002).

Anti-ulcer property:

Ethanolic leaves extract of *Tapinanthus dodoneifolius* grown on *Tamarindus indica* on aspirin induced gastric ulcer in wistar rats showed reduction in the incident of ulceration in a dose dependent manner (Baso and Mudi, 2017)

Anti-plasmodial, analgesic, anti-inflamatory and anti -pyretic activity:

Methanol whole plant extract of *Tapinanthus dodoneifolius* have been found to possess analgesic, anti-inflammatory and anti-pyretic activity anti-plasmodial activity against *Plasmodium berghei (*Abdullahi *et al.*, 2016)

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Plant material

The whole plant of *Tapinanthus dodoneifolius* was obtained from Sabon Gari Local Government Area of Kaduna State in June, 2018. It was identified by Mr. Namadi Sanusi of the Department of Botany, Ahmadu Bello University, Zaria by comparing with a specimen voucher number 0370 previously deposited in the herbarium.

3.1.2 Experimental animals

Swiss Albino mice of both sex (18-24g) as well asAdult Wistar rats of both sexes (110-156g) were used for the study. The animals were obtained from the Animal House of the Department of Pharmacology and Therapeutics Ahmadu Bello University Zaria. They were provided with rodent diet and water *ad libitum*. All experiments were carried out in compliance with Ahmadu Bello University Ethics on Animal Use and Care (ABUCAUC) with an approval number: ABUCAUC/2019/006.

3.1.3 Equipment and other materials

These are some of the equipment used during the course of the experiment: electronic weighing machine (Precision Electronic Instrument Company, India) water bath (Rajat Scientific Instrument, India)weighing balance, feeders, cages, dissecting kits(gold cross DS Malaysia), filter paper (whattman No 1), hand gloves, cotton wool, beakers, test tubes (Pyrex France), porcelain pestle and mortar, stop watch, spatula, desiccator, syringes and needles (1ml, 2ml, 5ml).

3.1.4 Chemicals and drugs

Apomorphine (Sigma-Aldrich, St. Louis, USA),Ketamine hydrochloride (Sigma-Aldrich, St. Louis, USA),Haloperidol (Sigma-Aldrich, St.Louis, USA), Distilled water, Amphetamine sulphate (Sigma Chemical Co, St Louis, USA),Diazepam (Roche Product Ltd., Welnyn Garden City, UK), Risperidone (Sigma-Aldrich, St. Louis, USA), methanol, Chloroform, Hydrochloric Acid (HCl), Sodium Hydroxide (NaOH), Molisch Reagent, Ferric Chloride Solution, 10% Ammonia Solution, Acetic Acid Anhydride, Dragendoff Reagent, Dettol (Disinfectant), Formalin and Normal saline (0.9% normal saline isotonic solution).

3.2 Methods

3.2.1 Preparation of plant material

The whole plant of *Tapinanthus dodoneifolius* was washed, air dried under shade then crushed into coarse powder with a pestle and mortar.The powdered plant material (900g) was extracted using cold maceration method with 7L of 70%$^{v}/_{v}$methanolfor three days with occasional stirring. The mixture was then filtered using Whatman filter No 1. The filtrate was concentrated and subjected to air drying in crucible. Percent (%) yield of the extract was calculated and the obtained extract was stored in a desiccator and subsequently referred to as Methanol extract of *Tapinanthus dodoneifolius* (METD). Fresh concentrations of the extract were prepared on each day of the experiment by reconstituting in distilled water.

The percentage extract yield was calculated as:

$$\frac{\text{Weight of extract}}{\text{Weight of dried powder}} \times 100$$

3.2.2 Phytochemical screening

Preliminary phytochemical screening of METD was carried out to identify the phytochemical constituents, using standard procedures.

3.2.2.1Test for carbohydrates (Molisch Test)

Few drops of Molisch reagent was added to a small portion of the extract (METD) in a test tube, and concentrated sulphuric acid was added down the side of the test tube to form a lower layer, a reddish-coloured ring at the interphase indicates the presence of carbohydrates(Evans, 2002).

3.2.2.2Test for anthraquinone (Bontrager's Test)

A small portion of the extract (METD) was placed in a dry test tube and 5 ml of chloroform was added and shaken for at least 5 minutes. This was filtered and equal volume of 10% ammonia solution was added to the filtrate and shaken. The presence of a bright pink colour in the aqueous (Upper) layer indicates free anthraquinones (Ayoola *et al.*, 2008).

3.2.2.3 Test for glycosides (Fehling's test)

To a portion of the extract (METD), 5 ml of dilute sulphuric acid was added and boiled on water bath for 10-15 minutes. The cooled mixture was neutralized with 20% KOH and then divided into two portions for reactions with fehling's solution and ferric chloride solution. To the first portion, 5 ml of a mixture of fehling's solutions A and B was added and boiled and the presence of a brick red precipitate indicates release of reducing sugar from hydrolysis of glycosides. To the second portion, about 3 ml of ferric chloride solution was added; a green to blue colour change indicates release of phenolic aglycones from hydrolysis glycosides. (Ayoola *et al*., 2008)

3.2.2.4 Test for steroids and triterpenes (Lieberman Bucchard Test)

A small portion of the extract (METD) was dissolved in 2 ml of acetic anhydride and 1 ml concentrated sulphuric acid was added down the side of the test tube to form a lower layer. Change of colour from violet to bluish-green was considered an indication for the presence of steroidal ring of the glycosides, while a reddish, pink or purple colour indicates the presence of triterpene(Evans, 2002).

3.2.2.5 Test for cardiac glycosides (Keller-Kiliani Test)

A small portion of the extract (METD) was dissolved in 1 ml of glacial acetic acid and with traces of ferric chloride solution and 1ml of concentrated sulphuric acid was added down the side of the test tube to form a lower layer at the bottom. Presence of a purple-brown ring at the interphase indicates the presence of deoxy-sugars and a pale green colour in the upper acetic acid layerindicates the presence of cardiac glycosides(Evans, 2002).

3.2.2.6 Test for saponins (Frothing Test)

About 10 ml of distilled water was added to a small portion of the extract (METD) and was shaken vigorously for 30 seconds. The tube was allowed to stand in a vertical position and was observed for 30 minutes. A honeycomb froth that persists for 10-15 minutes indicates presence of saponins(Evans, 2002).

3.2.2.7 Test for tannins (Ferric chloride Test)

Ferric chloride was added in drops of 3-5 to 0.5 g of the extract (METD) and the presence of a greenish-black precipitate indicates condensed tannins while a blue or brownish-blue precipitate indicates hydrolysable tannins. (Evans, 2002)

3.2.2.8 Test of flavonoids (Sodium hydroxide test)

Few drops of 10 % sodium hydroxide were added to a small portion of the extract (METD), yellowish coloration indicates the presence of flavonoids. (Evans, 2002)

3.2.2.9 Test for alkaloids

A small portion of the extract (METD) was stirred with 5 ml of 1% aqueous hydrochloric acid on a water bath and filtered. Ammonia solution was added to the filtrate followed with chloroform; this was gently shaken to allow separation. The chloroform layer was collected and dilute hydrochloric acid was added gently shaken to allow separation and the aqueous layer was divided into three portions. To the first portion, few drops of freshly prepared Dragendorff reagent was added and observed for formation of orange to brownish precipitates. To the second, one drop of Mayer's reagent was added and observed for formation of white to yellowish or cream color precipitates. To the third portion, 1ml of Wagner's reagent was added to give a brown or reddish or reddish-brown precipitates, which indicated the presence of alkaloids (Evans, 2002).

3.2.3 Acute toxicity study

The acute toxicity study was carried out based on Organization for Economic Co-operation and Development (OECD) guidelines 423 (2001) and fixed dose study was adopted with 5000 mg/kg weight of test animal as the limit dose. The guidelines suggest two type of acute oral toxicity tests; main and limit tests. Six animals were used (three per step). Three mice and rats eachwere fasted for 3-4 hours for rats while 1-2 hours for mice and each animal were administered 5000 mg/kg of methanol extract of *Tapinanthus dodoneifolius* based on the fasted body weight. Food but not water was withheld further for 1hour post extract administration. Each mouse was observed individually for the first 30 minutes and periodically during the first 4 hours and then daily for 14 days. The same procedure was repeated for another group of three mice. The animals were observed for signs and symptoms of toxicity, such as tremor, convulsion, salivation, lacrimation, diarrhoea,

lethargy, sleep, respiratory, behavioral pattern, time of onset of toxicity if any and length of recovery period as well as time of death.

3.2.4 Sub-chronic toxicity study

The guideline of OECD 407 (2008) was usedfor the study. Twenty four rats of both sexes were randomly divided into 4 groups of 6 animals each. The rats were administered with distilled water and extract at doses of 250, 500 and 1,000 mg/kg respectivelyorally for 28 consecutive days using orogastric cannula. Rats were maintained under standard conditions with food and water *ad libitum* for the entire period with close observation for signs and symptoms of toxicity. The body weights of the rats were taken weekly for 4 weeks. On day 29, animals were anaesthetised with chloroform and then euthanised. Blood samples were collected via cardiac puncture into plain bottles for serum biochemical analysis and into coagulated (EDTA) bottles for plasma haematological analysis. The organs such as kidney, liver, heart, lungs, stomach, and spleen were placed into 10% formalin fixative for histological examinations. Tissue slide were viewed at a magnification of x250 and photomicrographs of the tissues were obtained with the aid of a consultant histopathologist

The relative organ weight for each animal was calculated using the formular:

$$\frac{\text{Absolute organ weight}}{\text{Body weight of rats on sacrifice day (g)}} \text{X } 100$$

3.2.5 General Pharmacological Studies

3.2.5.1 Beam walking assay

The experiment was performed according to the method described by Stanley *et al.,* (2005) Mice were trained to walk along a ruler (80 cm long, 3 cm wide) elevated 30 cm above a bench by a metal support to a goal box. Three trials were done for each mouse, only the mice that showed no neurological deficit and walked successfully along the ruler was admitted into the study and further grouped into five groups of six mice each.Animals in group I received distilled water 10 ml/kg, group II-IV received methanol extract of*Tapinanthus dodoneifolius* (250mg/kg, 500 mg/kg and 1000 mg/kg respectively), while group V received diazepam 0.5 mg/kg. In the main test, each mouse was placed at one end of a cylindrical beam made up of wood (8 mm in diameter, 60 cm long and elevated 30 cm above a bench) and allowed to walk to the goal box.The number of foot slips, (one or both hind limb slipping from the beam) which is a sensitive measure of motor coordination deficit was recorded for each mouse using a tally counter.

3.2.6 Antipsychotic Studies

3.2.6.1 Apomorphine-induced stereotypic climbing behavior test in mice

The method described by Costall *et al.,* (1978) was adopted. Thirty Mice fasted overnight were randomly divided into five groups of six mice each. Animals in group I received distilled water 10 ml/kg, group II-IV received METD (250 mg/kg, 500 mg/kg and 1000 mg/kg respectively) while group V received Haloperidol 2mg/kg. One hour after administration, Apomorphine 1 mg/kg in 10% sodium metabisulphite was given *ip*, each mouse was placed in a cylindrical wire mesh cage with walls of vertical metal brass 2 mm diameter, 1cm apart, surmounted by a smooth surface. 30 mins after apomorphine treatment, each mouse was observed every 10 mins for thirty minutes and the climbing behaviour was recorded: four paws on the floor (0), one paw grasping on the vertical wall (1), fore paws

grasping on the vertical wall (2), four paws grasping on the vertical wall (3), the average climbing score was determined for each group of animals.

3.2.6.2 Swim-induced grooming test in mice

The method described by Chesher and Jackson (1981) was adopted. Thirty Mice fasted overnight were randomly divided into five groups of six mice each. Animals in group I received distilled water 10 ml/kg, group II-IV received METD (250 mg/kg, 500 mg/kg and 1000 mg/kg respectively) while group V received Haloperidol 2mg/kg per oral. Sixty minutes later, each mouse was placed individually in a 1000 mL beaker containing water at 25^{o}C up to 6 cm mark from the base for 3 min, each animal was removed and dried with towel for 30 seconds and placed immediately into rectangle perspex boxes. The total duration of grooming episodes in seconds was recorded for five minutes with a stop watch.

3.2.6.3 Ketamine-induced hyperlocomotion test in mice

This study was done according to the method described by Yamamoto *et al.,* (1997). Thirtymice were fasted for 12 hours and divided into five groups of six mice each. Group I received distilled water 10 ml/kg *p.o,* Group II, III, IV received graded doses of the extract at 250 mg/kg, 500 mg/kg, and 1000 mg/kg respectively while group V received Risperidone (0.5mg/kg). One hour after administration, all mice received 10 mg/kg Ketamine *i.p* and were placed individually in an open field arena measuring 40x20x18 cm which has visible lines drawn to divide the floor with frontal glass wall. One hour post administration of Ketamine, locomotive activity indicated by the duration of immobility and number of lines crossed was recorded for 5minutes using a stop watch.

3.2.6.4 Ketamine-enhanced immobility in forced swim test

The method described by Chindo *et al.,* (2012) was adopted. The mice were fasted for 12 hours and divided into six groups of five mice each. Group I received distilled water 10 ml/kg *p.o*, while groups II, III, IV, V and VI received 30mg/kg Ketamine *p.o* once daily for five days. One hour after the last treatment (5^{th} day) with ketamine for habituation, each mouse was placed in a standardized transparent glass cylinder (height 46cm, diameter 20cm) containing water at 25^{o}C to the depth of 30cm and was forced to swim for 5minutes (pretest session). On 6^{th} day, group II received distilled water10 ml/kg, while groups III, IV, V received graded doses of the extract at 250 mg/kg, 500 mg/kg and 1000 mg/kg while group VI was given risperidone (0.5mg/kg). Sixty minutes later, each animal was placed in the same glass cylinder containing water and forced to swim for 6 minutes. The immobility time was recorded for a period of 5 minutes (test session) after discarding activity in the first one minute, during which the animal tries to escape (Kroczka *et al.,* 2001) after each session, the mice was removed immediately from the cylinder, dried with a towel and kept in an open space until completely dried before returning the mice to their home cages.

3.2.6.5 Ketamine-induced schizophrenic like behaviour

The method described by Leucht *et al.,* (2003) was adopted, three groups of six mice each were grouped, group one received distilled water 10ml/kg while group two and three received effective dose of the METD (1000 mg/kg) and 0.5 mg/kg risperidone respectively for 14 days. From the 8^{th} day to the 14^{th} day, each group of animal received daily dose of 20mg/kg Ketamine, 30minutes after pre-treatment with distilled water, 1000 mg/kg of METD and risperidone. On the 15^{th}day, each group of mice were placed on Y maze, open

field and plexiglassbox apparatus for observation of cognitive behaviour, number of line crossing and social interaction respectively.

Open Field Test. The open field arena measuring 40x20x18 cm which has visible lines drawn to divide the floor with frontal glass wall. This apparatus was used to evaluate the exploratory activity of the animal. The observed parameter was the number of squares crossed (with all four paws).

Y-Maze Test. Spontaneous alternation performance was assessed using a Y-maze apparatus, which allows the evaluation of cognitive searching behavior. Each arm of the maze was 40 cm long, 25 cm high and 6 cm wide and converged to an equal angle. Each mouse was placed at the end of one arm and allowed to freely move through the maze during 8 min. The series of arm entries was recorded visually. An alternation was defined as entries in all three arms on consecutive occasions. The percentage of alternation was calculated as (total of alternations/total arm entries) − 2), as previously described (Dall'igna *et al.,* 2007; Yamada *et al.,* 1996).

Social Interaction Test. The testing apparatus consisted of a 60 × 40 cm Plexiglas box divided into three chambers. Mice were able to move between chambers through a small opening (6 × 6 cm) in the dividers. An iron cage in each of the two side chambers contained the probe mice. Test mice were placed in the center chamber. Mice were allowed 5 min of exploration time in the box, after which an unfamiliar, same-sex probe mouse from the same experimental group was placed in one of two restraining cages (Radyushkin *et al.,* 2009). The time spent in each of the three chambers was measured, and social preference was defined as follows: (% time spent in the social chamber) − (% time spent in the opposite chamber).

3.2.7 Determination of Side Effect of Potential Antipsychotic Agent

3.2.7.1 Catalepsy test in mice

The experiment described by Costall and Naylor (1974) was adopted. Thirty mice fasted for 12 hours, were randomly divided into five groups of six mice each. Animals in group I received distilled water 10 ml/kg, groups II-IV received METD (250mg/kg, 500mg/kg and 1000 mg/kg respectively) and group V received Haloperidol 2mg/kg per oral. Sixty minutes later, the test was done by gently placing the fore limbs of each animal on a horizontal plane wood surface (H= 6cm, W=4cm, L=16cm) and the duration of akinesia (period of time the animal remained in one position, before initiating any active movement) in seconds was recorded using a stop watch. An animal is considered cataleptic if it remained on the block for 60 seconds (Chatterjee *et al.*, 2012)

3.2.7.2 Vacuous chewing movement (VCMS) in rats

The method described by Naidu *et al.* (2003) was adopted. Thirty mice fasted for 12 hours, were randomly divided into five groups of six mice each. Animals in group I received distilled water 10 ml/kg, group II-IV received haloperidol (2 mg/kg) and METD (250mg/kg, 500mg/kg and 1000 mg/kg respectively) and group V received Haloperidol 2mg/kg *per oral* once daily for the period of 21 days. The behavioural assessment was carried out after 24 hours of last dose of haloperidol. On the test day, rats were placed individually in a small (30 x 20 x 30 cm) Plexiglas cage for the assessment of VCMs for the period of 5 minutes. VCMs were referred to as single mouth opening in the vertical plane not directed towards physical material (Naidu *et al.*, 2003).

3.3 Data Analysis

The parametricdata obtained were expressed as mean ± SEM. One way Analysis of Variance (ANOVA), Split Plot ANOVA (for weekly body weights) followed by Bonferonni multiple comparison (for level of significance between means) While non-parametric data were expressed as median. Kruskal Wallis test followed by Dunn's test for multiple comparisonwere used for the analysis. $p \leq 0.05$ was considered as level of statistical significance.

CHAPTER FOUR

4.0 RESULT

4.1 Percentage Yield of Methanol Extract of *Tapinanthus dodoneifolius*

The percentage yield of the pasty dark green methanol whole plant extract of *Tapinanthus dodoneifolius* was 14.4%$^{w}/_{w}$. the weight of the crude extract was 129.72g which was divided by 900g of the powered plant material.

4.2 Preliminary Phytochemical Constituents

The preliminary phytochemical screening of the methanol extract of *Tapinanthus dodoneifolius*revealed the presence of alkaloids, cardiac glycosides, saponins, tannins, flavonoids, steroids/triterpenes, terpenoids and carbohydrates. However, anthraquinones was absent (Table 4.1).

Table 4.1: Phytochemical Constituents Present in the Methanol Extract of *Tapinanthus dodoneifolius*

Phytochemical constituents	Inference
Alkaloids	Present
Cardiac glycosides	Present
Saponins	Present
Tannins	Present
Flavonoids	Present
Triterpenes/Steroids	Present
Terpenoids	Present
Carbohydrates	Present
Anthraquinones	Absent

4.3 Estimation of Median Lethal Dose of Methanol Extract of *Tapinanthus dodoneifolius*

The median lethal dose(LD_{50}) of the methanol extract of *Tapinanthus dodoneifolius*was estimated to be greater than 5000mg/kg body weight and there was no signs of toxicitysuch as salivation, sleep, tremor, lacrimation, convulsion, diarrhoea, lethargy, respiration and change in behavioural pattern and mortality.

4.4 Sub chronic Toxicity Profile of Methanol Extract of *Tapinanthus dodoneifolius*

4.4.1 Effect of Methanol Extract of *Tapinanathus dodoneifolius* on Body Weight (g) of Wistar Rats within28 days of Oral Administration

There was an inconsistent change in the weight of the animals at day 7 and 14and an increases weight gain at day 21 and 28 in all the groups. However, the animals that were treated with the extracts showed significant weight gain when compared to the control group. However, an increase in weight that was significant was observed in group treated with 500mg/kg at day 21 compared to the control (distilled water) in the study period (figure 1; Appendix 1)

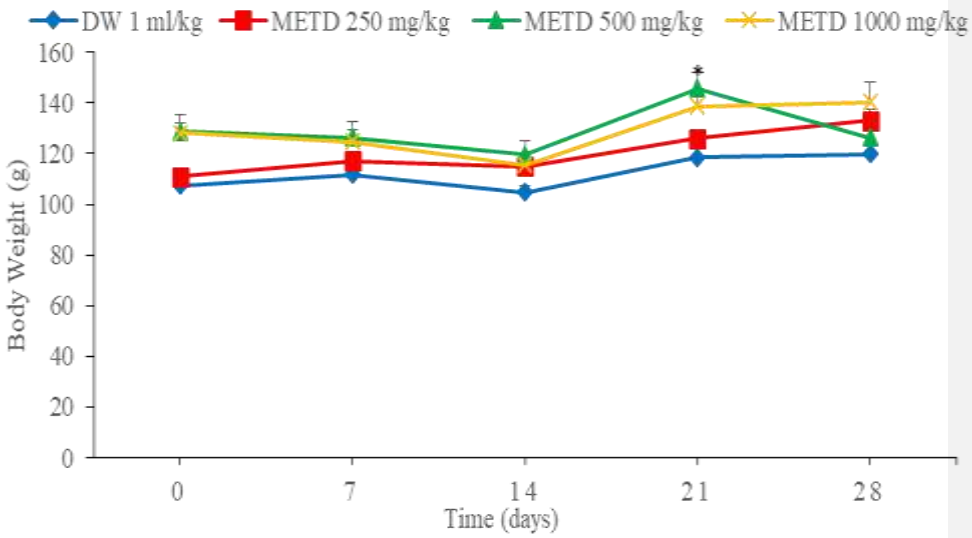

Figure 1: **Effect of Methanol Extract of *Tapinanathus dodoneifolius* on Body Weight (g) of Wistar Rats within28 days of Oral Administration**

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$ compared to distilled water. Split plot ANOVA followed by Bonferroni *post hoc* test, n=6, D/W-distilled water, METD = Methanolic Extract of *Tapinanthus dodoneifolius.*

4.4.2 Effect of Methanol Extract of *Tapinanthus dodoneifolius* onRelative Organ weights (%) in Wistar Rats within28 days of Oral Administration

The mean organ (kidney, heart, liver, lungs, stomach, and spleen) weights of the treated groups were not significantly (p>0.05) affected compared to the control group that receiveddistilled water at the dose of 10 ml/kg(Table 4.2)

Table 4.2:Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Relative Organ weights (%) in Wistar Rats within28 days of Oral Administration

Treatment		Relative organ body weight (%)					
(mg/kg)		Kidney	Heart	Liver	Lungs	Stomach	Spleen
D/W	1	0.74±0.05	0.42±0.04	3.78±0.14	0.97±0.19	1.28±0.12	0.55±0.10
ml/kg							
METD	1000	0.66±0.05	0.41±0.02	3.73±0.23	0.93±0.25	1.18±0.07	0.49±0.06
METD	500	0.63±0.04	0.36±0.02	3.41±0.13	0.69±0.06	1.21±0.10	0.45±0.03
METD	250	0.56±0.05	0.36±0.06	3.22±0.12	0.71±0.11	1.10±0.05	0.39±0.02

Values are expressed as Mean ± S.E.M;One way ANOVA followed by Bonferroni *post hoc* test, n=6, D/W-distilled water, METD=Methanol extract of *Tapinanthus dodoneifolius*,

4.4.3: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Liver Function Parameters in Wistar Rats within 28 days of Oral Administration

The METD did not produce any significant change in the liver function parameters (ALT, AST, TP, and ALB) at allthe doses administered. However, the highest dose (1000mg/kg) significantly produced a reduction in ALP (Table 4.3)

Table 4.3: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Liver Function Parameters in Wistar Rats within 28 days of Oral Administration

Treatment (mg/kg)	ALT (I.U/L)	AST (I.U/L)	ALP (I.U/L)	TP (g/dL)	ALB (g/dL)
D/W 1 ml/kg	13.00±3.08	143.20±18.83	35.24±4.77	9.22±0.68	3.16±0.18
METD 250	15.60±1.36	148.20±6.40	27.62±3.86	10.04±0.78	3.02±0.23
METD 500	9.50±3.07	156.17±8.45	26.03±2.52	12.33±1.01	2.90±0.09
METD 1000	9.00±1.53	95.00±13.95	20.48±1.96*	12.05±0.88	2.60±0.24

Values are expressed as Mean ± S.E.M., * = $p \leq 0.05$ compared tocontrol (D/W 1 ml/kg) One way ANOVA followed by Bonferroni *post hoc* test, n=6, ALT=Alanine amino transferase, AST= Aspartate amino transferase, ALP = Alkaline Phosphatase, TP = Total protein, ALB = Albumin, METD = Methanol extract of *Tapinanthus dodoneifolius*, IU = International unit, D/W-distilled water

4.4.4: Effect of Methanol Extract of *Tapinanthus dodoneifolius*on Serum Electrolyte and Renal Function Indices in Wistar Rats within 28 days Oral Administration

The methanol extract of *T. dodoneifolius* produced an inconsistent non-significant changes in the level of serum sodium, potassium, bicarbonate, urea, chloride and creatinine(Table 4.4).

Table 4.4: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Serum Electrolyte and Renal Function Indices in Wistar Rats within 28 days Oral Administration

Treatment (mg/kg)	Urea (mg/dl)	Creatinine (mEq/l)	Sodium (mmol/l)	Potassium (mmol/l)	Chloride (mg/dl)	Bicarbonate (mg/dl)
D/W 1 ml/kg	46.08±1.28	0.94±0.07	114.54±5.17	16.82±2.42	21.80±1.39	88.20±3.77
METD 1000	49.96±0.95	0.92±0.06	106.32±5.12	13.96±1.22	22.40±1.54	84.00±7.31
METD 500	44.05±1.62	0.88±0.07	123.55±3.08	17.00±1.06	25.33±1.91	85.83±2.40
METD 250	48.25±2.67	0.82±0.05	121.95±4.47	11.82±0.76	25.33±1.90	85.40±5.95

Values are expressed as Mean ± S.E.M;One way ANOVA followed by Bonferroni *post hoc* test, n=6, D/W-distilled water, METD = Methanol extract of *Tapinanthus dodoneifolius*

4.4.5: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on the Hematological Parameters of Wistar within28 days Oral Administration

Sub-chronic oral administration of the extract produced a statistical significant difference ($p \leq 0.05$) in granulocytes (GRAN) at doses of 500mg/kg and 1000mg/kg. Also, significant ($p \leq 0.05$) decrease in monocytes (MON) was observed at the dose of 1000 mg/kg (Table 4.5).

Table 4.5: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on the Hematological Parameters of Wistar Rats within 28 days Oral Administration

Treatment (mg/kg)	WBC ($\times10^3/\mu L$)	RBC ($\times10^6/\mu L$)	HGB (g/dL)	HCT (%)	PLT ($\times10^3/\mu L$)	LYMP (%)	MON (%)	GRAN (%)
DW 1 ml/kg	6.72±0.91	4.85±0.05	13.11±1.11	40.72±3.75	306.40±31.09	33.54±2.14	11.00±0.95	55.62±2.41
METD 1000	5.74±0.71	4.76±0.07	14.49±0.59	43.34±1.66	205.80±21.20	32.36±2.59	5.16±0.72*	44.64±1.52*
METD 500	5.27±0.89	5.07±0.31	13.03±0.69	39.98±2.09	323.47±34.93	32.47±1.93	7.18±1.67	44.85±2.83*
METD 250	6.10±0.77	4.65±0.14	13.33±0.46	41.52±1.05	400.67±25.72	36.92±2.51	9.13±1.24	53.15±2.36

Values are expressed as Mean ± S.E.M., * =$p \leq 0.05$ compared to D/W group - One way ANOVA followed by Bonferroni *post hoc* test, n=6, D/W = Distilled water, METD =Methanol extract of *Tapinanthus dodoneifolius*,WBC = White blood Cell, RBC = Red blood cells, HGB = Haemoglobin, HCT = Haematocrit, PLT = Platelets, LYMP = Lymphocytes, MON = Monocytes, GRAN = Granulocytes

4.4.6: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Histology of Some Organs of Wistar Rats after 28 days Oral Administration

There were no pathological changes in the heart muscles of rats in all treated groups. The kidney showed a non-dose dependent histological changes (slight tubular necrosis with lymphocyte hyperplasia). The highest dose of the treated group (1000mg/kg) showed a moderate hepatocyte necrosis while the 250 mg/kg and 500 mg/kg showed a normal hepatocytes. A dose dependent pathological changes (slight alveoli congestion, nuclei hardening and pyknosis) were seen in the lungs of the treated groups. A normal red and white pulp was observed at 250 mg/kg. However,lymphocyte hyperplasia was seen at dose 500 and 1000 mg/kg of treated group extracts). Similarly, normal gastric mucosa was observed at 250 mg/kg and 500mg/kg treated groups while lymphocyte hyperplasia in the animals treated with 1000mg/kg was observed. (Plate II to VI).

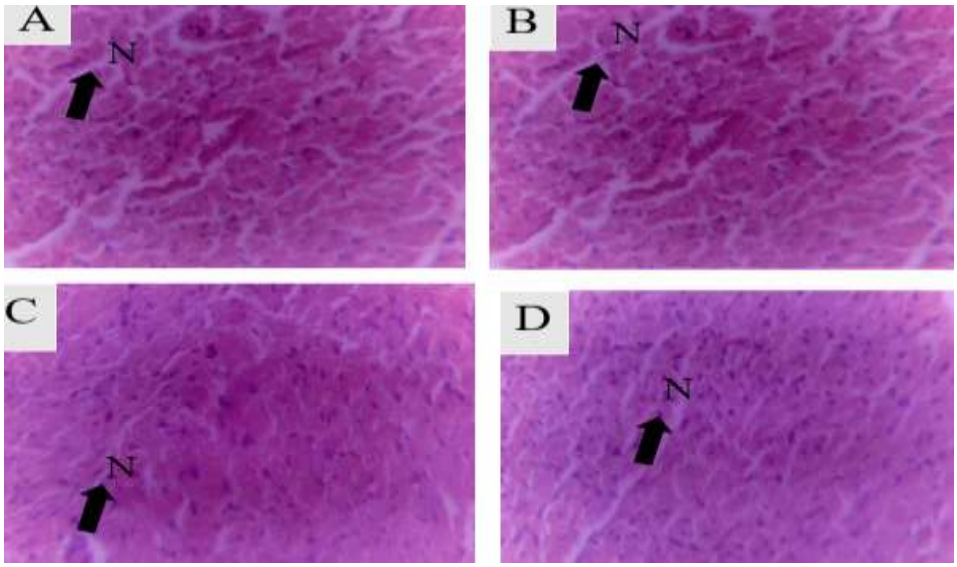

Plate II: Photomicrograph of heart sections showing normal cardiac muscles (N)(H & E ×250) of rats following 28 days oral administrations of Methanol Extract of *Tapinanthus dodoneifolius*: distilled water 10 ml/kg (A), 250 mg/kg (B), 500 mg/kg (C), 1000mg/kg(D)

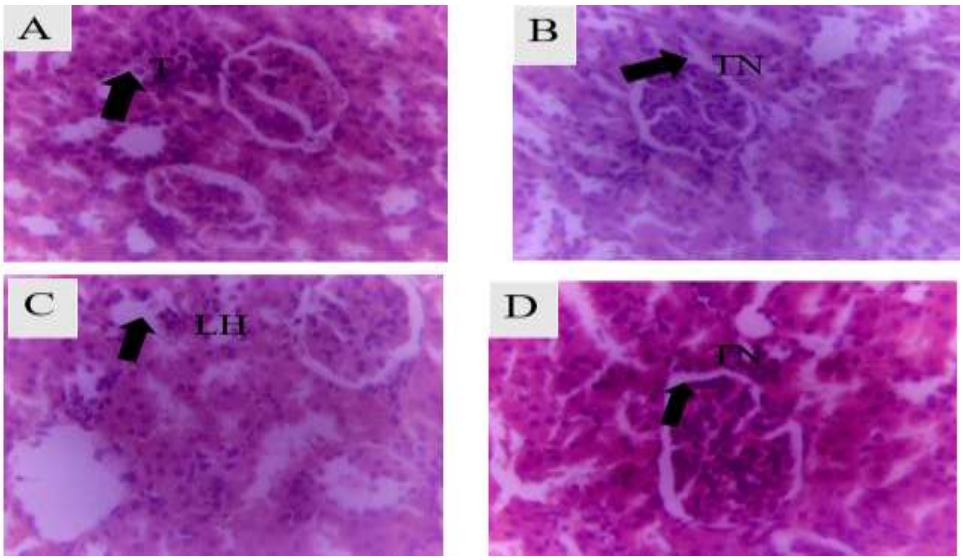

Plate III: Photomicrograph of kidney sections showing normal kidney tubules and glomerulus (T), slight tubular necrosis (TN), lymphocyte hyperplasia (LH) (H & E ×250) of rats following 28 days oral administrations of Methanol Extract of *Tapinanthus dodoneifolius*: distilled water 10 ml/kg (A), 250 mg/kg (B), 500 mg/kg (C), 1000 mg/kg (D)

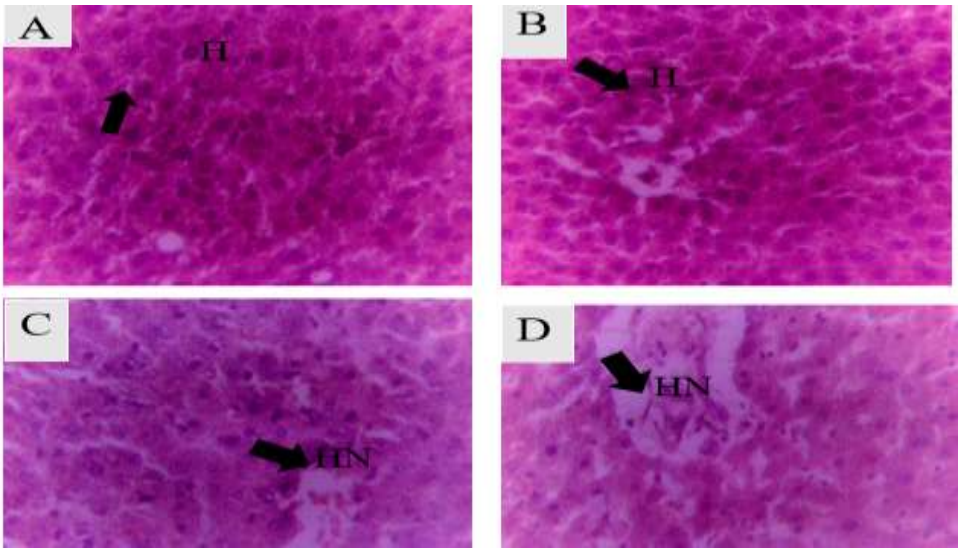

Plate IV: Photomicrograph of liver sections showing normal hepatocytes (H) and moderate hepatocyte necrosis (HN) (H & E ×250) of rats following 28 days oral administrations of Methanol Extract of *Tapinanthus dodoneifolius*: distilled water 10 ml/kg (A), 250 mg/kg (B), 500 mg/kg (C), 1000 mg/kg (D)

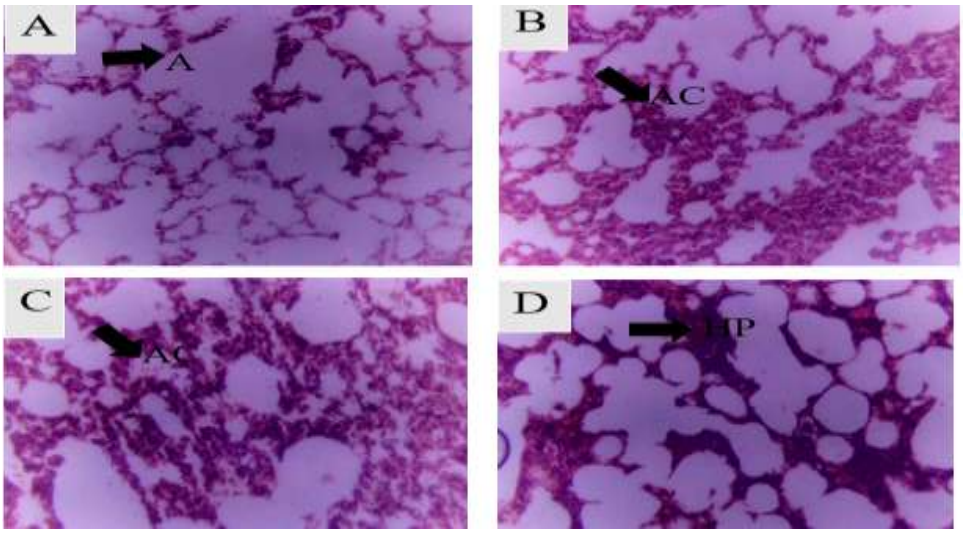

Plate V: Photomicrograph of lung sections showing normal lung alveoli (A), slight alveoli congestion (AC), nuclei hardening and pyknosis (HP) (H & E ×250) of rats following 28 days oral administrations of Methanol Extract of *Tapinanthus dodoneifolius*: distilled water 10 ml/kg (A), 250 mg/kg (B), 500 mg/kg (C), 1000 mg/kg (D)

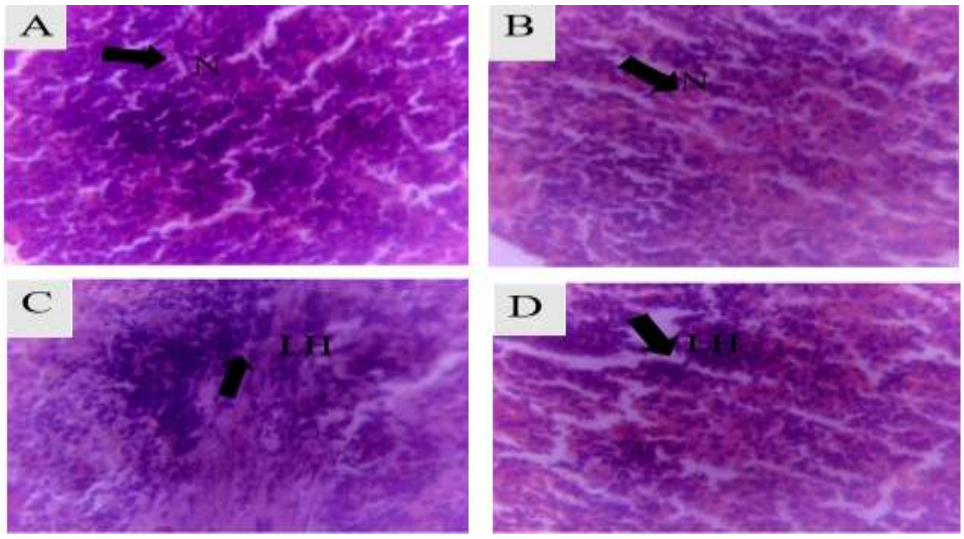

Plate VI: Photomicrograph of spleen sections showing normal red, white pulp (N), lymphocyte hyperplasia (LH)(H & E ×250) of rats following 28 days oral administrations of Methanol Extract of *Tapinanthus dodoneifolius*: distilled water 10 ml/kg (A), 250 mg/kg (B),500mg/kg(C),1000mg/kg(D)

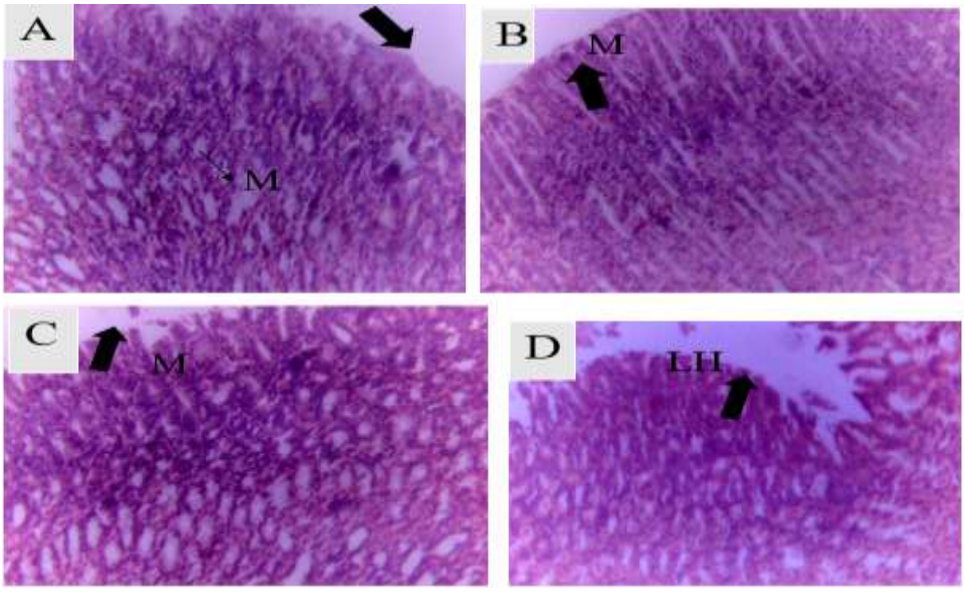

Plate VII: Photomicrograph of stomach sections showing normal mucosa epithelium of the stomach**(M) andlymphocyte hyperplasia (LH)(H & E ×250) of rats following 28 days oral administrations of Methanol Extract of *Tapinanthus dodoneifolius*:** distilled water 10 ml/kg (A), 250 mg/kg (B), 500 mg/kg (C), 1000 mg/kg (D)

4.5 General Pharmacological Studies

4.5.1: Effect of Methanol extract of *Tapinanthus dodoneifolius* on Motor Coordination in Mice

The extract at all the doses tested produced a non-significant (p>0.05) increase in the number of foot slips. However, diazepam (0.5mg/kg) significantly increased the number of foot slips (Figure 2; Appendix 2)

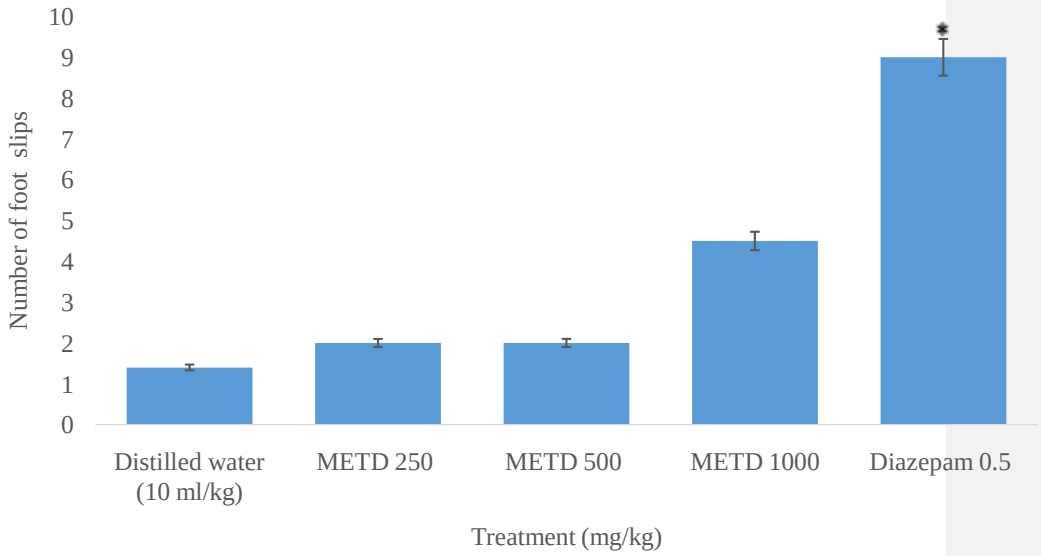

Figure 2: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Motor Coordination in Mice

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$ compared to distilled water (One way ANOVA followed by Bonferroni *post hoc* test) n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*

4.6 Antipsychotic Studies

4.6.1 Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Apomorphine-induced Stereotypic Climbing Behavior in Mice

On administration of Apomorphine to mice after one hour to induce stereotypic climbing behavior, mice in the negative control group (distilled water 10ml/kg) produced an increase in mean stereotypic climbing behavior. Mice treated with Methanol extract of *Tapinanthus dodoneifolius* at all doses (250, 500 and 1000mg/kg) showed a decrease in the mean climbing behaviour dose-dependently. Statistically, significant decrease ($p \leq 0.05$), ($p < 0.01$), ($p < 0.01$) in the mean climbing behaviour was observed in doses of 500mg/kg, 1000mg/kg and in the positive control group (Haloperidol 2mg/kg) respectively at the end of 30minutes study period when compared to the negative control group (distilled water 10 ml/kg) (Figure 3; Appendix 3)

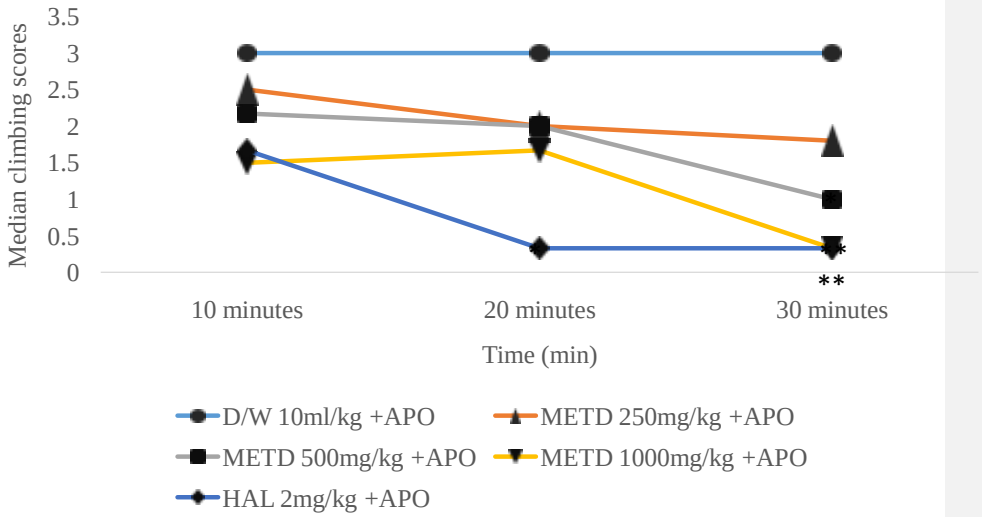

Figure 3: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Apomorphine-induced Stereotypic Climbing Behavior inMice

Values are expressed as Median; * = $p \leq 0.05$, ** = $p < 0.01$, compared to distilled water (Kruskal-Wallis test followed by Dunn's *post hoc* test) n=6, HAL= haloperidol, APO= apomorphine, METD = Methanol extract of *Tapinanthus dodoneifolius*

4.6.2: Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Swim-induced Groomingin Mice

The extract of *T. dodoneifolius* produced a significant ($p \leq 0.05$) dose dependent decrease in the mean grooming time. Similarly, Haloperidol (2mg/kg) showed a significant ($p < 0.001$) decrease in the mean grooming time in mice (Figure 4; Appendix 4)

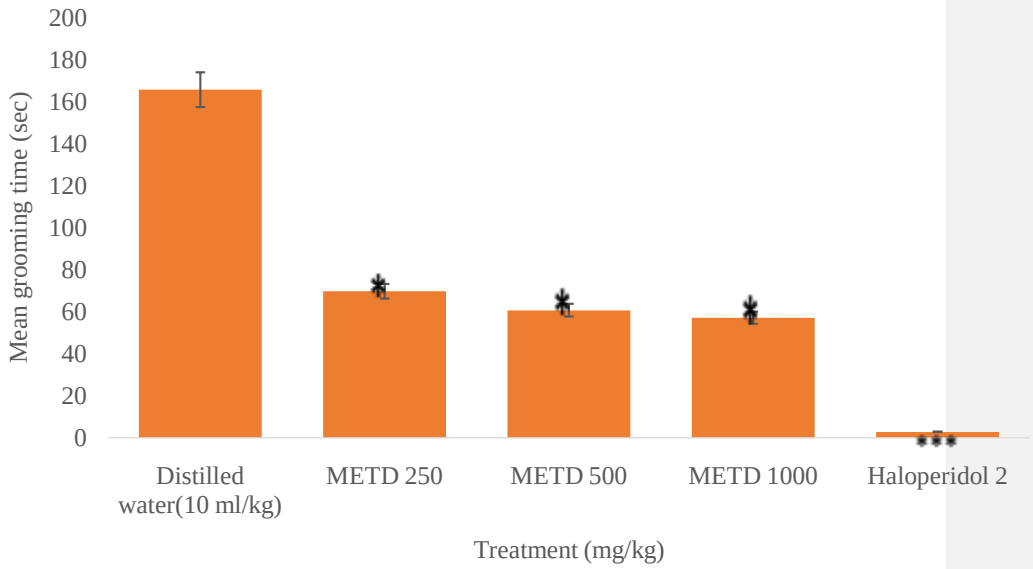

Figure 4: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Swim-induced Grooming in Mice

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$, *** = $p < 0.001$ compared to distilled water– One way ANOVA followed by Bonferroni *post hoc* test, n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*

4.6.3 Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Ketamine-induced Hyperlocomotionin Mice (Open Field Test)

The extract of *T. dodoneifolius* produced a non-significant dose dependent decrease in the number of lines crossed after induction with ketamine. However, risperidone (0.5mg/kg) significantly ($p \leq 0.05$) decrease the number of lines crossed post treatment with ketamine. The extracts significantly ($p<0.01$) but non dose dependently increased the immobility time induced by ketamine. Similarly, risperidne (0.5mg/kg) significantly ($p<0.001$) increased the immobility time in mice post ketamine treatment (Table 4.6)

Table 4.6: Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Ketamine-induced Hyperlocomotionin Mice

Treatment (mg/kg)	Number of lines crossed	Immobility time (Sec.)
D/W10ml/kg	101.83±24.39	16.17±3.52
METD 250	88.50±5.38	71.00±9.18**
METD 500	77.00±7.14	67.67±10.71**
METD 1000	62.00±10.66	73.33±7.10**
Risperidone 0.5	31.33±4.81**	125.83±11.46***

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$, ** = $p < 0.01$, *** = $p < 0.001$ compared to distilled water – One way ANOVA followed by Bonferroni *post hoc* test, n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*

4.6.4 Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Ketamine-Enhanced Immobility in Forced Swim Test

The extract of *T. dodoneifolius* at all the doses tested significantly ($p \leq 0.05$, $p < 0.01$) decreased ketamine induced enhanced immobility in mice. Similarly, risperidone (0.5mg/kg) showed a significant ($p < 0.01$)decrease in immobility time in mice (Figure 5; Appendix 5)

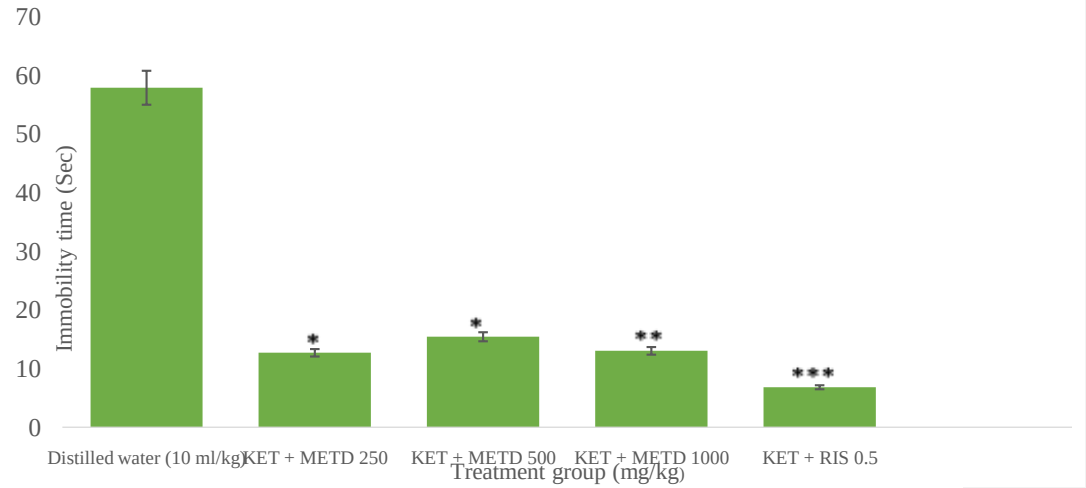

Figure 5: Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Ketamine-enhanced Immobility in Forced Swim Test

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$, ** = $p < 0.01$ compared to distilled water – One way ANOVA followed by Bonferroni *post hoc* test, n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*

4.6.5 Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Ketamine induced Schizophrenic like Behaviour in Mice

In the open field test, there was an insignificant decrease in the number of lines crossed in the animals treated with METD and risperidone compared to the control. In the Y-maze test, an insignificant increase in the percentage correction of alternation was observed in mice treated with METD and a significant (p<0.01) increase in the percentage correction of alternation was observed in mice treated with the standard drug (risperidone). In the social interaction test, an insignificant increase in the social preference was produced in the mice treated with METD and risperidone compared to the control group that were treated with distilled water (Table 4.6, 4.7 and 4.8)

Table 4.7: Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Line Crossing in Open Field Testin Mice

Treatment (mg/kg)	Number of lines crossed
Distilled water 10 ml/kg	82.60±18.09
METD 1000	60.60±10.75
Risperidone 0.5	36.20±12.53

Values are expressed as Mean ± S.E.M; No significant difference as compared to distilled water – One way ANOVA followed by Bonferroni *post hoc* test, n=5, METD = Methanol Extract of *Tapinanthus dodoneifolius*

Table 4.8: Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Percentage Correction of Alternation in Y-Maze Test in Mice

Treatment (mg/kg)	% Correction of alternation
Distilled water 10ml/kg	28.60±6.09
METD 1000	44.00±6.86
Risperidone 0.5	74.40±6.15**

Values are expressed as Mean ± S.E.M; ** = $p<0.01$ as compared to distilled water – One way ANOVA followed by Bonferroni *post hoc* test, n=5, METD = Methanol Extract of *Tapinanthus dodoneifolius*

Table 4.9: Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Social Preference of Mice in Social Interaction Test

Treatment (mg/kg)	Social preference (Sec.)
Distilled water 10 ml/kg	-1.80±96.78
METD	58.60±153.37
Risperidone	281.60±81.22

Values are expressed as Mean ± S.E.M; No significant difference as compared to distilled water – One way ANOVA followed by Bonferroni *post hoc* test, n=5, METD = Methanol Extract of *Tapinanthus dodoneifolius*

4.6.6 Effect of Methanol Extract of *Tapinanthus dodoneifolius*on Catalepsy

The methanol extract of *T. dodoneifolius* did not produce a significant change in the duration of catalepsy at all the doses tested. However, Haloperidol (2mg/kg) significantly ($p \leq 0.05$) increased the duration of catalepsy compare with the group treated with distilled water (Figure 6; Appendix 6)

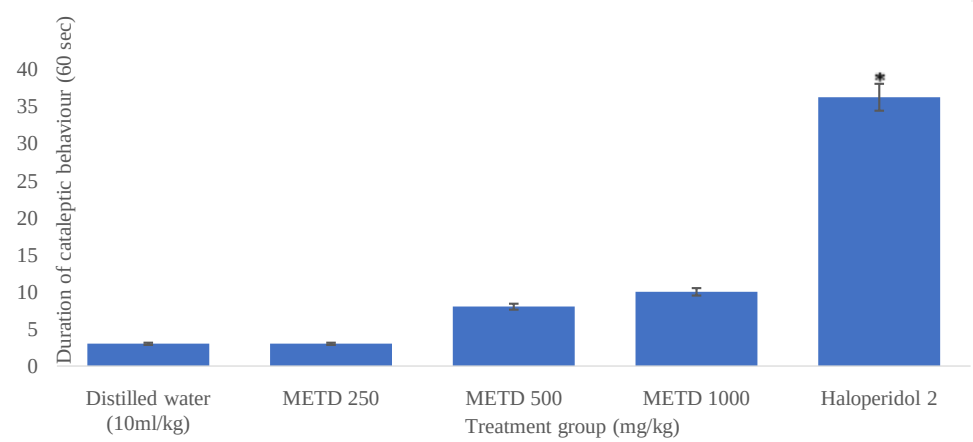

Figure 6: Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Catalepsy in Mice

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$ compared to Distilled water – One way ANOVA followed by Bonferroni *post hoc* test, n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*

4.6.7: Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Haloperidol-induced Vacuous Chewing Movementsin Rats

The methanol extracts of *T.dodoneifolius* at all the doses (250, 500, 1000 mg/kg) tested produced a dose dependent but non-significant increase in the vacuous chewing movement. However, haloperidol 2mg/kg significantly ($p \leq 0.05$) produced an increase in the number of vacuous chewing movement compared to the control. (Figure 7; Appendix 7)

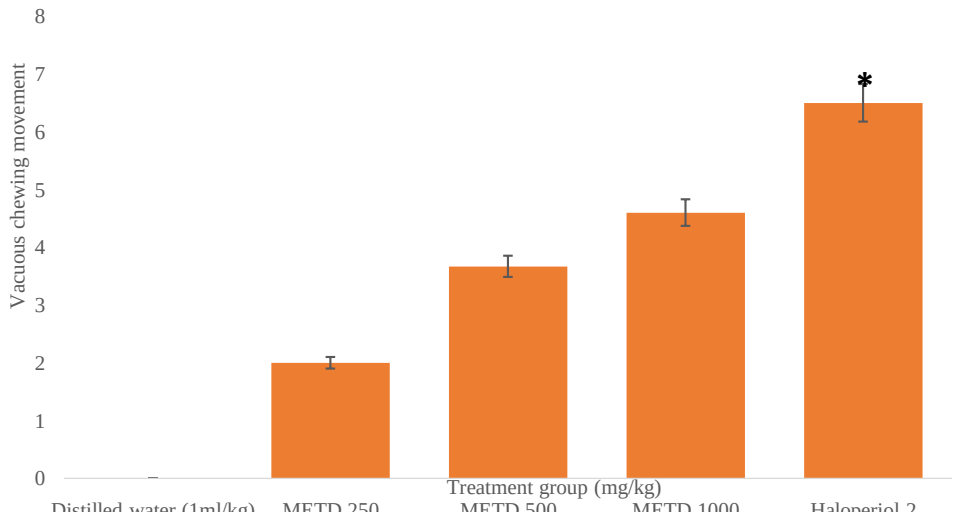

Figure 7: Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Haloperidol-induced Vacuous Chewing Movementsin Rats

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$ compared to Distilled water group – One way ANOVA followed by Bonferroni *post hoc* test, n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*

CHAPTER 5

5.0 DISCUSSION

Tapinanthus dodoneifolius DC Danser (Loranthaceae) also known as African mistletoe is a medicinal plant and has diverse ethno-medical uses including the management of mental illness, epilepsy, stomach ache, diarrhoea, dysentery, diabetes, hepatitis, malaria, wound, hypertension and cancer (Deeni and Sadiq, 2002; Ndamitso *et al.*, 2017). This study therefore attempts to validate the traditional claims of the use of the plant in the management of mental illness as well as establishing the sub-chronic toxicity profile of the plant (*Tapinanthus dodoneifolius*).

Preliminary phytochemical screening of the methanol whole plant extract of *Tapinanthus dodoneifolius* revealed the presence of alkaloids, cardiac glycosides, saponins, tannins, flavonoids, steroids, terpenoids and carbohydrate. This result concurs with the work of Abdullahi *et al.,* 2016 and Ndamitso *et al.,* 2017 which reported the presence of similar constituents in the plant (*T. dodoneifolius*). The presence of these phytochemicals such as alkaloids, glycosides, saponins, triterpenoids and flavonoids may be responsible for the observed biological activity of the plant (Ayanwuyi *et al.,* 2016). Triterpenoids which was observed in METD have been shown to have CNS depressant activity (Amos *et al.,* 2002)

The median lethal dose (LD_{50}) of methanol whole plant extract of *Tapinanthus dodoneifolius*which was estimated to be greater than 5000 mg/kg, suggest that the extract is relatively safe on acute administration (OECD, 2002)

Reduction in body weight and internal organ weights are simple and sensitive indices of toxicity after exposure to a substance (Witthawaskul *et al.,* 2003). The observed increase in

body weight of the animals administered with METDcould possibly be attributed to the nutritive constituents in the extract (Ezeonwumelu *et al.*, 2011)

Liver function tests provide in depth information about the state of the liver describing its functionality (example are albumin, total protein), cellularintegrity (e.g. amino transaminase) and its link with the biliary tract (gamma-glutamyltransferase and alkaline phosphatase) (Adeoye and Oyedepo, 2004). Liver contains a host of enzymes such as ALT, AST and ALP. The activities of these enzymes are used to assess the functional status of the liver and as biochemical markers of liver damages (Moss and Ralph 1999). Hepatotoxic drugs causes damage to the liver cell membrane and these enzymes are leaked out into serum and shows increase activities (Kumar *et al.*, 2004). A rise in plasma alkaline phosphatase (ALP) level is usually a characteristic finding in cholestatic liver disease (Kaneko, 1989), the significant reduction in ALP at the highest dose (1000mg/kg) of the METD shows that no possible cholestasis occured. METD did not produce any significant changes in the level of ALT and AST compared to the control.

Serum total protein and albumin assay are used as reliable and sensitive indicators of liver function status since they are synthesized and metabolized in the liver (Ganong, 2001). Estimation of the total protein is one of the most widely used means of measuring hepatocellular injury (Ighadaro *et al.*, 2015). It reflect nutritional status and maybe used to screen for and diagnose kidney disease, liver diseases and other disease conditions (Ighadaro *et al.*, 2015). The serum total protein and albumin levels were not affected by the extract administration, suggesting that the METD did not interfere with the protein synthetic capacity of the liver.

Renal dysfunction and kidney failure can be assayed by continuous measurement of urea and creatinine. As the kidneys become impaired, creatinine level in the blood rises due to damage to the functional nephrons and consequently poor clearance by the kidneys (Gnanamani*et al.,* 2008). Thus, abnormally high levels of serum creatinine and urea are biomarkers of possible malfunction of the kidneys (Asmawi *et al.*, 2016). In this study both urea and creatinine levels were normal in the extract treated groups when compared to the controls. The function of the electrolytes (sodium, potassium, chloride and bicarbonate) help regulate nerves and muscle function and maintain acid-base balance and water thus, since there was no significant difference between the treated groups and the control, it suggest that the extracts at the treated doses did not alter the electrolytes function, in maintaining fluid balance among compartments and does not seem to interfere with renal function and that the renal integrity was preserved (Kaneko, 1989).

Evaluation of haematological parameters can be used to assess the deleterious effect of foreign compound including plant extract on the blood (Agbaje *et al.*, 2009; Ibrahim *et al.*, 2016). Such investigation is necessary as changes in the blood system have higher predictive value for human toxicity when data are extrapolated from animal studies (Olson *et al.*, 2000). Non-significant effect of the METD on RBC, HCT, Hb, and PLT shows that the plant does not affect erythropoiesis and osmotic fragility of the red blood cells (Guyton and Hall, 2006). The white blood cells (WBC) are the first line of defence which responds to infectious agents, inflammatory process or tissue injury. Monocytes and granulocytes are type of white blood cells that play important role in the defence against infection and inflammation, a significant decrease in the level of monocytes and granulocytes at 1000 mg/kg observed in the present study suggest infection or inflammation.

The histological examination of body organs is one of the golden standards for evaluating treatment related pathological changes (OECD, 2008). The heart showed a normal cardiac muscles in all the doses of METD, while the morphology of liver, kidney, lungs, spleen and stomach were slightly altered. The histological examination of METD of the liver of the rats treated with METD showed hepatocytes necrosis at the highest dose (1000 mg/kg), this could have led to the significant decrease in the level of ALP of 1000 mg/kg dose of METD which suggest inhibition or inactivation of the enzyme by the extract, which led to little protein synthesis.

The glomerulus is the site of removal of several chemicals and it may be injured by any toxic substances (Himri *et al*., 2011). The presence of the METDin the kidney of the treated animals could have been responsible for the mild distortion of tubular cells in the kidney. The alveolar congestion and nuclei hardening of the lungs produced by the highest dose (1000 mg/kg) of METD, may have caused a delay in the absorption of oxygen and other gaseous substances required by the body since rapid absorption of the alveolar epithelium is desirable for diffusion of gaseous substances into the pulmonary capillaries for normal body functions. The stomach is responsible for the digestion of food, secretion of gastric juices as well as mucus which helps to coat its lining, preventing erosion by gastric juices and secretion of gastric hormones. Lymphocytes hyperplasia was observed at the 500 mg/kg METDwhich may be due to inflammatory cells infiltration in the gastric, suggesting a protective response by the tissues. The spleen acts as a filter for purifying blood, removing microbes and worn out damaged red blood cells, it is an important organ in the immune system, producing the white blood cells that fight infection and synthesize antibodies. Slight lymphocytosis seen in the spleen maybe due to long exposure of the highest dose (1000

mg/kg) of (METD) which might have led to spleen tissue promoted activation and hyperplasia of lymphocytes in the spleen. METD may have enhanced the function of the spleen to maximize the elimination of toxins in the body and maintain the body balance.

The beam walking assay is a test for pharmacologically-induced motor coordination deficits, and is predictive of sedative effects (Stanley *et al.,* 2005). This assay has been found to detect benzodiazepine-induced motor coordination deficits, with the number of foot slips being a sensitive measure of motor coordination deficits (Stanley *et al.,* 2005). The METD produced a non-significantly increase in the number of foot slips, which suggestthat the depressant effect of the METD might be centrally mediated and not due to peripheral muscular blockade (Stanley *et al.,* 2005).

Apomorphine is a non-selective dopamine receptor agonist which activates D_2 and to a lesser extent D_1 receptors (Guardia *et al.,* 2002). It increases the intensity and duration of stereotypic behaviours by acting on post synaptic dopamine D_2 receptors (Stolk and Reck, 1970). Decrease hyperactivity and stereotypy due to the inhibition of apomorphine, suggests interference with central dopaminergic neurotransmission (Potter and Hollister, 2004). Hyperactivity of the dopaminergic mechanisms of the nigrostariatal and mesolimbic systems due to apomorphine, causes stereotype behaviour and hyperactivity (Herman *et al.*,1985). The ability of a drug to antagonize apomorphine induced stereotypic behaviour has been correlated with antipsychotic activity (Protals *et al.,* 1976; Costall *et al.,* 1978; Yaro *et al.,* 2007). In this study, the METD dose dependently reduced the apomorphine-induced stereotypic climbing behaviour and was significant at 30^{th} minute on observation, suggesting antidopaminergic activity. Similarly, haloperidol which is a standard antipsychotic

agentshowed a significant decrease in the mean climbing behaviour in the mice from the 20th minutes of observation.

The swim-induced grooming test is also one of the model used for screening of antipsychotic agents, the grooming behaviour induced by immersion in water involves mainly dopamine D_1 receptors (Van *et al.,* 2010). This behaviour is inhibited in a dose dependent manner by inhibitors of dopamine receptors(Ingale and Kasture, 2012). The METD produced a significant dose dependent decrease in the mean grooming time in the animals which suggests that the METD may have a potential D_1 dopaminergic antagonistic activity. Similarly, haloperidol which is a standard antipsychotic agent showed a significant decreases in swim induced grooming in the animals.

Ketamine, at sub-anaesthetic doses produces hyperactivity which is closely related to the psychotic agitation seen in patients with psychosis (Chatterjee *et al.,* 2012). It may influence dopamine transmission and receptor activation via multiple mechanisms, biochemical data have shown that ketamine enhance dopamine release and inhibit dopamine uptake in the striatum and cortex respectively (Irifune *et al.,* 1991; Benturquia*et al.,* 2008; Chatterjee *et al.,* 2012). Another mechanism by which ketamine might produce this adverse behavioral effects is related to the blockade of NMDA receptors located on inhibitory GABAergic neurons in the limbic and subcortical brain regions(Duncan *et al.,* 1998). This disinhibitory action has been reported to increase the neuronal activity and excessive dopamine release in the limbic striatal region (Moghaddam *et al.,* 1997). The study showed that the METD significantly decreased hyperactivity (hyperlocomotion) induced by ketamine in a dose-dependent manner, suggesting that the plant possesses antipsychotic effect.

Ketamine-enhanced immobility in forced swim test in rodent is one of the acceptable animal model for the test of negative symptoms of schizophrenia (particularly the depressive feature): it shows a state of 'despair' in mice and decrease in immobility time serves as a specific and selective index of antidepressant activity (Porsolt, 2001; Chindo *et al.,* 2012). The METD produced a significant decrease in ketamine enhanced immobility in the FST which suggests antipsychotic property of the extract and previous studies have reported the involvement of 5-hydroxytryptaminergic (5-HT) system in the negative symptoms of schizophrenia (Chatterjee *et al.,* 2012) The findings by Chindo *et al.,* (2012) suggested that ketamine-enhanced immobility in the FST might be mediated, at least in part, via 5-HT_{2A} receptors blockade, since 5-HT_{2A} receptor blockers such as risperidone decreases the ketamine-enhanced immobility time. Since, the METD showed a significant reduction of ketamine-enhanced immobility in FST similar to risperidone, it further suggest that the extract may possess antipsychotic property that may attenuate the negative symptoms of schizophrenia.

Ketamine induced schizophrenic like behaviour despites positive and negative symptoms as well as cognitive impairments induced by ketamine (Hou *et al.,* 2013) have been partially attributed to the blockade of NMDARs. Indeed, the blockade of NMDARs located on inhibitory GABAergic neurons in the limbic and subcortical brain regions leads to an increase in neuronal activity in the limbic-striatal circuits through an increase in glutamate and dopamine release; these neurochemical events relate to the positive symptoms of schizophrenia (Chatterjee *et al.,* 2012; Javitt and Zukin, 1991; Lorrain *et al.,* 2003). The blockade of NMDARs in the ventral tegmental area (VTA) promotes a decrease in dopamine release in the PFC, which may be partially responsible for the negative and

cognitive symptoms (Takahata and Moghaddam, 1998; Seamans and Yang, 2004;Neill *et al.,* 2010). Therefore, schizophrenia is associated with strongly interconnected abnormalities of glutamatergic and dopaminergic transmission (Laruelle *et al.,* 1996) that are at least in part reproduced by the chronic administration of ketamine in mice (Chatterjee *et al.,* 2012). From the result, it demonstrated that the administration of METD reversed ketamine-induced hyperlocomotion (test being correlated with positive symptoms of schizophrenia) with results comparable to risperidone, an atypical antipsychotic. These results call for attention to an involvement of dopaminergic neurotransmission in METD antipsychotic effects. The study also showed that METD reversed ketamine-induced alterations in the Y-maze test, suggesting an effect of the METD against ketamine-induced deficits in working memory. Social withdrawal is one of the core negative symptoms of schizophrenia (Lysaker *et al.,* 2012). In this study METD was able to reverse the decreases in the percentages of social contacts induced by ketamine. Indeed, the negative and cognitive symptoms of this mental disorder have been related to microglial activation and the resulting inflammatory response (Monji *et al.,* 2009). In this regard, METD effects on these symptomatic dimensions may partly result from its anti-inflammatory properties as well as effects on glutamate neurotransmission

The catalepsy test is frequentlyused in drug screening to evaluate the liability of potential antipsychotics to induce extrapyramidal side effects based on increased duration of catalepsy. These side effects are suggested to be from decreased dopamine activity based on blockade of dopaminergic neurotransmission, preferably D_2 receptors. However, the test for catalepsy demonstrated that the METD is devoid of catalepsy on the animals based on a non-significant increase in the duration of catalepsy.

Vacuous chewing movement (VCM)test in rats is one of the most widely used and acceptable model for the study of side effects of antipsychotic agents and the main parameter evaluated is the presence of orofacial dyskinesias (Turrone *et al.,* 2002). Chronic treatment of rats with haloperidol showed increased frequencies of VCMs as compared to control animals. The administration METD did not produce significant increase in the vacuous chewing movements compared to the control (distilled water).

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

The methanol whole plant extract of *Tapinanthus dodoneifolius*have been shown to possess significant antipsychotic properties at the doses tested, providing some scientific rationale for the use of the plant traditionally in mental illness. The METD was found to be relatively non-toxic at median lethal dose (greater than 5,000 mg/kg). However, the study suggests that sub-chronic administration of the METDdid not possess significant toxic effects that could affect its medicinal use.

6.2.1 Recommendations

Based on the knowledge acquired in the course of this research, the following recommendations are proposed:

1. Further studies should be carried out to fractionate, isolate and characterized the bioactive principles of the extract responsible for the observed antipsychotic activities.
2. Chronic studies should be carried out in order to ascertain the toxic effect of the extract on long term treatment
3. Evaluation of food and water intake should be carried out since the extract treated rats showed inconsistent weight gain compared to the control rats

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APPENDICES

Appendix 1:Methanol Extract of *Tapinanthus dodoneifolius* on Body Weight of Wistar Ratswithin 28 days of Oral Administration

	Treatment (mg/kg)			
Days	D/W 10ml/kg	250mg/kg	500mg/kg	1000mg/kg
Day 0	107.50±0.85	111.00±1.93	128.67±6.70	128.17±3.84
Day 7	111.67±2.23	117.17±2.32	126.33±6.28	124.67±4.97
Day 14	104.83±2.54	115.00±4.26	119.50±5.73	115.60±4.55
Day 21	118.50±3.81	126.00±3.58	145.67±6.31*	138.60±6.79
Day 28	119.80±5.00	133.17±4.11	126.33±5.98	140.40±8.15

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$ compared to distilled water. Split plot ANOVA followed by Bonferroni *post hoc* test, n=6, D/W-distilled water, METD = Methanolic Extract of *Tapinanthus dodoneifolius.*

Appendix 2: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Motor Coordination in Mice

Treatment (mg/kg)	Number of foot slips
Distilled water 10mg/kg	1.40±0.24
METD 250	2.00±0.37
METD 500	2.00±0.32
METD 1000	3.40±0.68
Diazepam 0.5	9.00±3.47*

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$ compared to distilled water (One way ANOVA followed by Bonferroni *post hoc* test) n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*

Appendix 3: Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Apomorphine-induced Stereotypic Climbing Behaviour inMice

Treatment (mg/kg)	Mean Climbing Behavior		
	10 Min	20 Min	30 Min
D/W10ml/kg	3.00±0.00	3.00±0.00	3.00±0.00
METD 250	2.50±0.50	2.00±0.71	1.80±0.73
METD 500	2.17±0.48	2.00±1.00	1.00±0.63*
METD 1000	1.50±0.67	1.67±0.61	0.33±0.03**
Haloperidol 2	1.67±0.61	0.33±0.03*	0.33±0.21**

Values are expressed as Median; * = $p \leq 0.05$, ** = $p < 0.01$, compared to distilled water (Kruskal-Wallis test followed by Dunn's *post hoc* test) n=6, HAL= haloperidol, APO= apomorphine, METD = Methanol extract of *Tapinanthus dodoneifolius*

Appendix 4:Effect of Methanol Extract of *Tapinanthus dodoneifolius* on Swim-induced Grooming in Mice

Treatment (mg/kg)	Mean grooming time (Sec.)
Distilled water 10ml/kg	165.40±22.25
METD 250	69.60±30.85*
METD 500	60.60±11.66*
METD 1000	57.00±15.54*
Haloperidol 2	2.80±1.07***

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$, *** = $p < 0.001$ compared to distilled water – One way ANOVA followed by Bonferroni *post hoc* test, n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*

Appendix 5:Figure 5: Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Ketamine-enhanced Immobility in Forced Swim Test

Treatment (mg/kg)	Immobility time (Sec.)
Distilled water 10ml/kg	57.80±13.35
METD 250	12.67±8.29*
METD 500	15.40±5.91*
METD 1000	13.00±6.16**
Risperidone 0.5	6.80±1.95**

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$, ** = $p < 0.01$ compared to distilled water – One way ANOVA followed by Bonferroni *post hoc* test, n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*

Appendix 6:Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Catalepsy in Mice

Treatment (mg/kg)	Duration of catalepy (60 Sec.)
Distilled water 10mg/kg	3.00±0.00
METD 250	3.00±0.58
METD 500	8.00±0.58
METD 1000	10.00±4.00
Haloperidol 2	36.20±9.87*

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$ compared to Distilled water – One way ANOVA followed by Bonferroni *post hoc* test, n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*

Appendix 7:Effect ofMethanol Extract of *Tapinanthus dodoneifolius* on Haloperidol-induced Vacuous Chewing Movementsin Rats

Treatment (mg/kg)	Vacuous chewing Movements
Distilled water 1 ml/kg	0.00±0.00
METD 250	2.00±0.37
METD 500	3.67±0.42
METD 1000	4.60±1.44
Haloperidol 2	6.50±0.96*

Values are expressed as Mean ± S.E.M; * = $p \leq 0.05$ compared to Distilled water group – One way ANOVA followed by Bonferroni *post hoc* test, n=6, METD = Methanol extract of *Tapinanthus dodoneifolius*