

**SOME NEUROPHARMACOLOGICAL STUDIES ON ETHANOL EXTRACT
AND FRACTIONS OF *TAPINANTHUS GLOBIFERUS* A. RICH MISTLETOE
ON *VITELLARIA PARADOXA* HOST IN LABORATORY ANIMALS**

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**A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE
STUDIES, AHMADU BELLO UNIVERSITY, ZARIA NIGERIA IN PARTIAL
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PHARMACOLOGY**

**DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS
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SEPTEMBER, 2018

DECLARATION

I declare that the work in this thesis entitled “Some Neuropharmacological Studies on Ethanol Extract and Fractions of *Tapinanthus globiferus* A. Rich Mistletoe on *Vitellaria paradoxa* Host in Laboratory Animals” has been carried out by me in the Department of Pharmacology and Therapeutics under the supervision of Prof. (Mrs.) H. O. Kwanashie, Prof. N. M. Danjuma and Prof. A. M. Musa.

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

Mustapha Huguma Abdullahi

Date _____

CERTIFICATION

This thesis entitled “SOME NEUROPHARMACOLOGICAL STUDIES ON ETHANOL EXTRACT AND FRACTIONS OF *TAPINANTHUS GLOBIFERUS* A. RICH MISTLETOE ON *VITELLARIA PARADOXA* HOST IN LABORATORY ANIMALS” by Mustapha, Huguma ABDULLAHI meets the regulations governing the award of the degree of Doctor of Philosophy in Pharmacology of the Ahmadu Bello University, Zaria, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my late parents.

ACKNOWLEDGEMENTS

I owe my gratitude to the Almighty Allah by Whose will, power and wisdom this thesis was successfully completed.

I wish to express my sincere gratitude and appreciation to my supervisors, Prof. (Mrs.) Helen O. Kwanashie, Prof. Nuhu M. Danjuma and Prof. Aliyu M. Musa for their useful suggestions, advice and constructive criticism throughout this work.

I would like to express my appreciation to those who showed concern, interest and gave moral, physical, spiritual and material resources as well as time in seeing to the completion of this work, may God bless you all.

I am also grateful to my family, particularly my beloved wife; Maijidda Ibrahim and my children for their prayers, understanding and moral support.

May Allah bless you all abundantly. Thank you.

ABSTRACT

Tapinanthus globiferus is a semi-parasite mistletoe which grows on branches of large number of trees including *Vitellaria paradoxa*. It used locally in the management of insomnia, epilepsy, anxiety, headaches and hypertension. This study evaluated the toxicity and neuropharmacological properties of ethanol extract and fractions of *T. globiferus*. These were carried out using standard methods. Phytochemical screening of *T. globiferus* ethanol extract (TgE) revealed presence of alkaloids, anthraquinones, flavonoids, cardiac glycosides, saponins, steroids terpenoids and tannins variously distributed among the fractions. The intraperitoneal LD₅₀ of TgE was 1,300 and 3,800 mg/kg in mice and rats respectively and >5,000 mg/kg orally. However, fractions of ethylacetate and ethylacetate water insoluble intraperitoneal LD₅₀ were 1,400 and 1,100 mg/kg respectively, and 3,800 mg/kg each for butanol and residual aqueous in mice. Oral administration of TgE for 28 days in rats produced no effects on body weight and serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and total protein at 87.5, 175 and 350 mg/kg, but at 350 mg/kg, serum levels of total bilirubin and conjugate bilirubin were significantly ($p \leq 0.05$) elevated while packed cell volume, haemoglobin and red blood cell were decreased. TgE produced significant ($p \leq 0.05$) decreases in serum urea, creatinine and sodium concentrations, and increases in potassium and chloride concentrations at 175 and 350 mg/kg, whereas bicarbonate concentrations were unaffected at all doses compared to 1.0 ml/kg normal saline control. Liver sections at 175 and 350 mg/kg showed distortion and degeneration of hepatocytes but no toxic effects at 87.5 mg/kg. Kidney and spleen architecture were normal at 87.5 and 175 mg/kg, but 350 mg/kg resulted in degeneration of Bowman's capsule, tubular degeneration and blood vessel distractions in kidney, as

well as degeneration in red pulp and necrosis of epithelial cells in spleen sections ($\times 100$ H and E). TgE and fractions significantly ($p \leq 0.05$) decreased onset and increased duration of diazepam-induced sleep but ethylacetate insoluble fraction had no effect at tested doses compared to control. Ethylacetate fraction (EF) was the most active among the fractions but produced no effect in ketamine-induced sleep at all tested doses. TgE and EF were significantly ($p \leq 0.05$) reduced number of head dips in hole-board at tested doses. However, bicuculline at 5 mg/kg increased number of head dips which were antagonised by EF and diazepam at 300 and 2 mg/kg respectively. TgE produced significant ($p \leq 0.05$) increased in number of foot slips in beam-walk at 350 mg/kg and EF at 300 mg/kg, while BF showed no activity at 250, 500 and 1,000 mg/kg compared to control. TgE produced no effect in elevated plus-maze and number of entries and time spent in open arms at 175 and 350 mg/kg. TgE did not exhibit effect in number of rearing at tested doses but at 350 mg/kg reduced number of steps climbed in staircase. However, TgE significantly ($p \leq 0.05$) reduced number of lines crossed and rearing at 175 and 350 mg/kg respectively in open field and offered significant effect in duration of haloperidol-induced catalepsy at 350 mg/kg, apomorphine-induced climbing behaviour at 87.5 and 175 mg/kg and no effect in duration of immobility in tail suspension at all doses compared to control. Antiseizure activities tested showed that TgE had no effect on onset of seizure compared to control in PTZ, STN, and PRT-induced seizures but offered 16.67, 33.33 and 50 % protection against PTZ and PRT at 87.5, 175 and 350 mg/kg. However, TgE did not protect mice and chicks against STN- and MES-induced seizures respectively. The study showed that extract and ethylacetate fraction of *T. globiferus* are less toxic and contained active constituents which have sedative effects and minimum anticonvulsant properties.

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ABBREVIATIONS

α	Alpha
β	Beta
γ	Gamma
$^{\circ}\text{C}$	Degree Celsius
%	Percent
μ	Micro
5-HT	5-Hydroxytryptamine
ALP	Alkaline Phosphatase
ALT	Alanine aminotransferase
ANOVA	Analysis of Variance
AST	Aspartate aminotransferase
Baso	Basophils
BCL	Bicuculline
BD	Blood vessel Distraction
BF	Butanol Fraction
BZDs	Benzodiazepines
CB	Conjugate Bilirubin
CHCl_3	Chloroform
Cl⁻	Chloride ion
Cm	Centimetre
CNS	Central Nervous System
CSD	Central Square Duration
CSE	Central Square Entries

CTZ	Chemoreceptor Trigger Zone
D₁	Dopamine sub receptor type 1
D₂	Dopamine sub receptor type 2
DB	Degeneration of Bowman's capsule
DH	Degeneration of Hepatocytes
DL	Decilitre
DR	Degeneration in Red pulp
DZP	Diazepam
E	Eosin
EF	Ethylacetate Fraction
EIF	Ethylacetate Insoluble Fraction
EPM	Elevated Plus-maze
Eosi	Eosinophils
G	Gram
GABA	Gamma Amino Butyric Acid
GAD	Generalized Anxiety Disorder
H	Haematoxylin
Hb	Haemoglobin
HCO₃⁻	Bicarbonate
HF	Hexane Fraction
HLTE	Hind Limb Tonic Extension
H₂O	Water
Hz	Hertz
IMP	Imipramine

<i>i.p.</i>	Intraperitoneal
K⁺	Potassium ion
Kg	Kilogram
L	Litre
LC	Line Crossed
Lab	Laboratory
Ltd	Limited
Lymp	Lymphocytes
LD₅₀	Median Lethal Dose
MAOI	Monoamine Oxidase Inhibitor
mA	Micro Amp
MD	Moderate Distortion
MEST	Maximal Electroshock Test
min	Minutes
mg	Milligram
ml	Millilitre
mm	Millimetre
mmole	Millimole
Mono	Monocytes
Na⁺	Sodium ion
NAPRI	National Animal Production and Research Institute
NE	Necrosis of Epithelial cell
Neut	Neutrophils
NMDA	N-methyl-D-aspartate

nm	nanometre
NS	Normal Saline
PBT	Phenobarbitone
PCV	Packed Cell Volume
PNS	Peripheral Nervous System
PNT	Phenytoin
PRT	Picrotoxin
PTZ	Pentylentetrazole
Plc	Public limited company
p.o	per oral
RBC	Red Blood Cell
RF	Residual Fraction
Rr	Rearing
SEM	Standard Error of Mean
S.C	Subcutaneous
Sec.	Seconds
SPSS	Statistical Package for Social Science
STN	Strychnine
SV	Sodium Valproate
T.	<i>Tapinanthus</i>
TB	Total Bilirubin
TD	Tubular Degeneration
TgE	<i>Tapinanthus globiferus</i> Ethanol Extract
TP	Total Protein

UK	United Kingdom
UNO	United Nation Organization
USA	United State of America
WBC	White Blood Cell
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

Neuro-pharmacological agents induce changes in the function of cells of the nervous system. However, nervous system is the most important physiological part that differentiate human from other species. Disorders of nervous system function may lead to malfunction of other systems, which are the major concern to human society, and a field in which pharmacological intervention plays a key role. Drugs acting on the central nervous system (CNS) were among the first to be discovered by primitive humans and are still the most widely used group of pharmacological agents (Nicol, 2007). Plant products or extract may produce similar pharmacological activity with orthodox medicine on CNS. However, substances that can be able to act on CNS may selectively relieve pain, depression, anxiety, insomnia and other neuropsychiatric and neurological disorders (Pandy *et al.*, 2012). Herbalists use leaves, flowers, stem and root barks of plants to prevent, relieve and treat illnesses and also to improve one's sense of wellbeing, but the mechanisms by which herbal medicine acts in the CNS have not been elucidated. However, herbal medicines are believed to be more potent, safe and affordable.

1.1 Statement of the Research Problem

Most people in the world have experienced at least one neuropsychiatric or neurological problem during their lifespan (WHO, 2017a). However, several new drugs for the treatment of neurological and neuropsychiatric disorders have been put into clinical practice, but up to one third of psychiatric patients remain resistant to optimum drug treatment (Kwan and Brodie, 2000) and only few individuals can afford to buy the

drugs (Nisar *et al.*, 2011). Insomnia is a significant public health problem which affects large segment of the population at one point or another in life (Morin and Jarrin, 2013). Some of the drugs used in insomnia such as diazepam, midazolam and flurazepam are drugs of tolerance, dependence and abuse potential (Charles and Robert, 1997).

Depression is a serious mood disorder which affects most population worldwide (Nisar *et al.*, 2011). Nigeria was ranked the most depressed in Africa in which 3.9% of the population suffered from depression and 2.7% of the population suffered from anxiety disorder (WHO, 2017b). However, depression and anxiety disorders cost the global economy one trillion US dollar each year (WHO, 2016).

Despite the improvements and other developments in the treatment of schizophrenia and other neuropsychiatric disorders, schizophrenia still the most devastated neuropsychiatric disorder that affect men and women in the world (Ramesh and Reddy, 2014). Nigeria with a population of about 170 million has about 1.7 million people suffering from schizophrenia (Adegbaju, 2014) and nearly 80% of them live in low and middle income countries (WHO, 2017a).

Herbalists use plant remedies in the management of neuropsychiatric and neurological disorders (Andrews, 1982). WHO and UNO have acknowledged the fact that, about 80% of world rural population relies on herbal medicine for the management of psychiatric and other neurological problems but about 40% of the problems have remained unresolved and 60% of plants used lack scientific merit (WHO, 2013). However, the problems associated with herbal medicine include, lack of scientific

proof, unpredictable dosage, intangible practices of traditional medicine and possibly improper diagnosis of ailment among others (Sofowora, 1993).

1.2 Justification of the Study

Most of the people in rural areas use plants as their source of medicine. Plants might form a virgin field for research work, but focus is yet to receive the attention of researchers (Sofowora, 1993). Among these plants are *Tapinanthus globiferus* that grows on *Vitellaria paradoxa* tree which has great medicinal importance and is used in traditional medicine for the management of neuropsychiatric and neurological disorders.

Tapinanthus globiferus is used in traditional medicine of Northern Nigeria for the treatment of insomnia, epilepsy, anxiety and headache (personal communication) but its effectiveness needs to be investigated scientifically.

Neuropharmacological study of ethanol extract and fractions of *T. globiferus* in rats, mice and chicks may provide a justification for the traditional uses and also may provide an important source for the development of better and safer drugs for the treatment of neuropsychiatric disorders (e.g. depression, schizophrenia, insomnia) or neurological (e.g. epilepsy, alzheimers, stroke, parkinsonism).

1.3 Theoretical Framework

The screening process of medicinal agents usually starts with index of acute toxicity studies (LD₅₀). The result of the LD₅₀ determination enabled the conduct of specific

behaviour tests to evaluate the neuropharmacological properties of the extract and fractions of *Tapinanthus globiferus*.

1.3.1 Toxicity study

Toxicity study is conducted first to investigate the acute and sub-acute toxicities of ethanol extract of *T. globiferus*. The index of acute toxicity (LD₅₀) of the plant is determined using Lorke (1983) method. This method can be used for every route of administration and it uses fewer animals to obtain adequate information on the acute toxicity. However, the result of acute toxicity study enabled the selection of range of doses used in conducting specific behavioural tests. Sub-acute toxicity is conducted to further observe the toxicity or safety of the extract. The method of Hodge and Sterner (1943) was adopted for sub-acute toxicity study. The observation and analysis of haematological, biochemical and histopathological components or toxicity index of the extract were carried out using acceptable techniques.

1.3.2 Diazepam-induced sleep test

Diazepam-induced sleep test is used to illustrate central nervous system (CNS) properties of drugs. The pharmacological tests of sedative, hypnotics, tranquilizers, neuroleptics as well as antidepressants are based on the potentiation of sleeping time induced by other sedative agents (Vogel, 2008). However, analeptics and stimulants shorten sleeping time. In this study, diazepam-induced sleep in mice is used as described by Rakotonirina *et al.* (2001). Diazepam acts at the level of the limbic, thalamic and hypothalamic regions of the CNS through potentiation of gamma amino butyric acid (GABA). GABA is known to be an important inhibitory neurotransmitter in

the brain. GABA interacts with GABA-receptors (GABA_A, GABA_B and GABA_C-receptor). GABA_A-receptor controls the opening of chloride channel for the entering of chloride anions resulting in the neuronal hyperpolarisation (Mirshafa *et al.*, 2013).

1.3.3 Ketamine-induced sleep test

Ketamine-induced sleep is used to evaluate antidepressant-like effects (Razmjou *et al.*, 2016). Ketamine is a widely used anaesthetic that exerts its depressant effect by reducing neuronal excitation via N-methyl-D-aspartate (NMDA) receptor as a non-competitive antagonist and mediating sympathetic responses. Besides the direct action of ketamine on NMDA receptor, it also affects dopaminergic, noradrenergic, cholinergic and serotonergic neurotransmission (Manocha *et al.*, 2001).

1.3.4 Hole-board test

This method is employed to evaluate certain components of behavior in mice such as curiosity or exploration (Crawley, 1985). It has been accepted as an experimental animal model for the evaluation of psychosis, sedation and anxiety condition (Crawley, 1985; Goodman *et al.*, 2006). Poking the nose into a hole is a typical behavior of mice and indicating a certain degree of curiosity. An agent that decreases this parameter reveals a more sedative property (Mandal *et al.*, 2001). Hence, anxiolytics have been shown to increase the number of head dips (Takeda *et al.*, 1998). Benzodiazepines tend to suppress nose-poking at relatively low doses (Vogel, 2008).

1.3.5 Mouse beam walking assay

The method of beam walking assay is used to evaluate the activity of drugs that interfere with motor coordination and is more sensitive than the rota-rod in determining benzodiazepines-induced motor coordination deficits (Stanley *et al.*, 2005). However, the method has increased ability in predicting doses of extract and diazepam that cause sedation (Vogel, 2008) and increase foot slips in mice reveals sedative activity of the extract or drugs.

1.3.6 Elevated plus-maze test

Elevated plus-maze is regarded as a reliable measure of anxiolytic activity. It has been proposed for selective identification of anxiolytic and anxiogenic drugs. Anxiolytic drugs increase the number of entries into open arms and the time spent in the open arms whereas anxiogenic agents do the opposite (Vogel, 2008). Benzodiazepines and valproate decrease motor activity and increase open arm exploration time. The method was validated by Lister (1987) and it was adopted for this research.

1.3.7 Elevated staircase test

This model was first described by Thiebot *et al.* (1973); it evaluates anxiolytic activity of chemical agents. The modified model of Simiand *et al.* (1984) was used in this study. The model can determine the effects of psychotropic agents on rearing and climbing separately and can detect behavioural effects of agents active at the GABA_A receptor (Simiand *et al.*, 1984). Mouse staircase test is an efficient paradigm for studying agents active at the GABA_A receptor complex (Weizman *et al.*, 2001). Step-climbing is

claimed to reflect exploratory or locomotor activity, while rearing behavior is an index of anxiety state (Vogel, 2008).

1.3.8 Open field test

The Open Field Test provides simultaneous measures of locomotion, exploration and anxiety. The model is usually used as measures of locomotor activity, but also measures of exploration and anxiety. A high frequency of these behaviours indicates increased locomotion and exploration and lower level of anxiety. The number of central square entries and the time spent in the central square are measures of exploratory behaviour and anxiety. A high duration of these behaviours indicates high exploratory behaviour and low anxiety levels.

1.3.9 Haloperidol-induced catalepsy test

Haloperidol-induced catalepsy test is used to measure the reduced ability to initiate movement and the failure to achieve correct posture in mice, which is assessed by means of a standard bar test. Mice are considered to be cataleptic if they remain on posture position for 30 seconds or more (Salam, 2011). Catalepsy is a sign of extrapyramidal effect of drugs that inhibit dopaminergic transmission or increase histamine release in brain (Pathan *et al.*, 2009). Haloperidol is a dopamine D₂-receptor antagonist (Pandy *et al.*, 2012). Haloperidol induces catalepsy by blocking the postsynaptic striatal D₁- and D₂-receptors (Klemm, 1993). The phenomenon of catalepsy can be used for measuring the efficacy and the potential side effects of neuroleptics (Vogel, 2008). Neuroleptics which have an inhibitory action on the nigrostriatal dopamine system induce catalepsy (Costall and Naylor, 1974) while

neuroleptics with little or no nigrostriatal blockade produce relatively little or no cataleptic behaviour (Honma and Fukushima, 1976).

1.3.10 Apomorphine-induced climb test

Apomorphine administration produced a peculiar climbing behaviour in mice which is characterized initially by rearing and then spontaneous climbing activity (Costall *et al.* 1978). The ability of a drug to antagonize apomorphine-induced stereotyped climbing behaviour in the mouse has been correlated with neuroleptic potential (Protais *et al.*, 1976, Costall *et al.*, 1978). Reduction or suppression of climbing behaviour in mice after apomorphine administration can be used for neuroleptic drugs evaluation (Vogel, 2008).

1.3.11 Tail suspension test

This test is sensitive to antidepressant drugs such as tricyclic, monoamine oxidase inhibitors (MAOI), serotonin specific reuptake inhibitors and atypical antidepressant (Porsolt *et al.*, 1977; Steru *et al.*, 1985). Tail suspension was found to be an easy method to evaluate potential antidepressant compounds (Vogel, 2008). Stressed mouse would become immobile after an initial period of struggling to escape. This immobility signifies behaviour that resembles a state of mental depression which can be reduced by antidepressant drugs (Porsolt *et al.*, 1977; Steru *et al.*, 1985). However, several mouse strains are essentially resistant to tail-suspension induced immobility (Vogel, 2008).

1.3.12 Pentylenetetrazole-induced convulsion test

The test is used primarily for screening antiepileptic drugs. However, it has been shown that most anxiolytic agents are able to prevent or antagonize metrazol-induced convulsions. Stimulant, antidepressant, neuroleptic and some antiepileptic drugs do not show metrazol-antagonism at tolerable doses (Lippa *et al.*, 1979). Tonic extension of the hind limbs is an indication of epilepsy in mice but absence of clonic spasms in the 30 minutes observation period indicated the compounds have ability to abolish the effect of pentylenetetrazole (PTZ) on seizure threshold (Swinyard *et al.*, 1989). PTZ-induced seizures are further characterized into different patterns that are one or more generalized myoclonic body twitches, generalized body seizure with loss of righting reflex, loss of righting reflex with tonic forelimb extension and loss of the righting reflex with tonic forelimb and hind limb extensions (Loscher *et al.*, 1991). Pentylenetetrazole exert its convulsive effect by inhibiting the activity of gamma amino butyric acid (GABA) at GABA_A receptor. Sodium valproate is effective in the treatment of myoclonic and absence seizures (McNamara, 2006) and active against both pentylenetetrazole and maximal electroshock seizures.

1.3.13 Strychnine-induced convulsion test

The test is used to screen agents that are active in the central nervous system (CNS) as well as chemical that induced seizure. The convulsing action of strychnine is due to interference with postsynaptic inhibition mediated by glycine (Vogel, 2008). Glycine is an important inhibitory transmitter to motor neurons and inter-neurons in the spinal cord, and strychnine acts as a selective, competitive antagonist to block the inhibitory effects of glycine at all glycine receptors (Rajendra *et al.*, 1997). Strychnine-sensitive

postsynaptic inhibition in higher centres of the CNS is also mediated by glycine. Compounds which reverse the action of strychnine have been shown to have anxiolytic properties (Costa *et al.*, 1975) as well as effective anti-epileptics (Larson, 1969). Phenobarbitone, valproate and diazepam are effective against strychnine-induced convulsion.

1.3.14 Electroshock-induced convulsion test

The electrically induced seizures are useful models for detecting compounds with anticonvulsant activity. Administration of maximal electroshock in chicks through corneal electrodes or ear-clip electrodes in mice is used to deliver the stimuli. Tonic hind-limb extensions are evoked by electric stimuli which are suppressed by anti-epileptics such as phenytoin, carbamazepine and phenobarbitone (Vogel, 2008). The behavioural and electrographic seizures generated in this model are consistent with the human disorder (Swinyard *et al.*, 1989).

1.3.15 Picrotoxin-induced seizure

Picrotoxin-induced convulsions are used to further evaluate CNS-active compounds (Vogel, 2008) and a model of recurring focal epilepsy screening (Freitas *et al.*, 2006). Picrotoxin is regarded as a GABA_A receptor antagonist that produces seizures by blocking the chloride ion channels linked to GABA_A receptor complex (Nicoll, 2007). Drug effective against picrotoxin-induced convulsion includes phenobarbitone and diazepam.

1.3.16 Phytochemical screening

The relationship between phytochemical constituents and pharmacological activity has been shown by many authors. Previous studies revealed that, chemical constituents such as alkaloids, flavonoids, saponins and tannins obtained from plants produce pharmacological activity (Kporou *et al.*, 2010; Bassey, 2012; Ajao and Akindele, 2013).

1.3.17 Statistical analysis

Analysis of data generated and significant differences using One-way Analysis of Variance (ANOVA) followed by Dunnett's *post-hoc* test gives portable presentation of results as tables and figures.

1.4 Aim and Objectives

1.4.1 Aim

The aim of this research is to study some neuropharmacological effects of the ethanol extract and fractions of *Tapinanthus globiferus* that grows on *Vitellaria paradoxa* (host) in rats, mice and chicks with a view to find out the most active fraction.

1.4.2 Objectives

The objectives of the study are:

- i. To identify the phytochemical constituents present in ethanol extract and fractions of *T. globiferus* using standard methods.
- ii. To determine safety (through acute and sub-acute toxicity studies) of the ethanol extract of *T. globiferus* in mice and rats using oral and intraperitoneal routes of administration.

- iii. To evaluate some neuropharmacological properties such as sedative, anxiolytic, antipsychotic, antidepressant and anticonvulsant of ethanol extract of *T. globiferus* in mice and chicks.
- iv. To determine fractions of ethanol extract of *T. globiferus* and identify the most active among them.
- v. To investigate the possible pathway and mechanisms of the most active fraction.

1.5 Statement of Research Hypothesis

The ethanol extract and fractions of *T. globiferus* contain active phytochemical constituents which have some neuropharmacological activities.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Toxicity Study

One of the basic goals of researchers in their effort to discover new drugs is to develop new products with high therapeutic efficacy and low toxicity profile. The detailed toxicological assessment should form a critical component of both early and late phases of drug development from medicinal plants to avoid toxicity tragedies after such drugs might have been approved for therapeutic purposes (Kolawole *et al.*, 2013). One of the effective ways to determine the toxicity profile of herbal preparations is to assess their effects on haematological and biochemical parameters as well as histological features of organs. However, observation of physical parameters in rats may provide the safety or toxicity of extract. Extracts are considered to be toxic when they caused damage to the blood cells or organs structure of the experimental animals. Some researchers have attributed the toxicity of extract and other pharmacological properties of a number of plants to their chemical constituents (Calixto *et al.*, 2000; Ezenwali *et al.*, 2010; Abdullahi *et al.*, 2014).

2.1.1 Haematology

Many diseases are as a result of damage to blood cell. This can be evaluated through haematological indices such as red blood cells, haemoglobin, packed cell volume and white blood cell differentials. Hence, study of these indices or profiles would help to provide valuable information for diagnosis, treatment and monitoring of certain diseases. Haematological complications consist mainly of abnormalities in the function,

morphology and metabolism of erythrocytes (red cell) and leukocytes (white cell) (Sakuljaitrong *et al.*, 2012).

2.1.2 Biochemistry

Many food as well as extract obtained from plants possessed toxic compounds at certain stages unless processed. The toxicity of some plants may be due to the presence of cyanogenic glycosides or lectins (Daisy *et al.*, 2009). Several plant extracts have been examined for use in a wide variety of disorders, while others have been reported to cause injury to the organs (Atawodi *et al.*, 2011). Measurement of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP) and total bilirubin (TB) levels served as a means for the indirect assessment of liver damage. Study in laboratory animal has shown that increases in the levels of ALT, AST, ALP, TP and TB in the serum are associated with liver damage (Daisy *et al.*, 2009). Hence, the amount of ALT, AST, ALP, TP and TB in the blood is directly related to the extent of the liver damage. Most of the waste products are excreted through kidney and the consequent impairment of kidney functions usually results in raised blood levels of urea, creatinine, and electrolytes such as sodium, potassium, bicarbonate and chloride (Contran *et al.*, 2005).

2.1.3 Histopathology

The extent to which an organ is susceptible to toxicity depends on its nature. For example the kidney, liver and spleen are more highly vascularised making them more susceptible to toxicity than the bone tissues. Liver is the main organ involved in biotransformation of most of the xenobiotics in the body (Dadzeasah, 2012). Most of

the bi-products of medicinal plants in the body are excreted primarily by the kidneys and some are nephrotoxic because they can affect the functional and structure integrity of the kidney. Spleen is an organ that serves as a reservoir of erythrocytes and change in morphology of spleen may affect the level of erythrocytes which lead to red cell disorders notably anaemia (Choudhury and Sinha, 2015). Organs that present cytoarchitectural distortions are of histopathological importance (Yunusa, 2014).

2.2 Drugs that Acts on the Central Nervous System

Action of drugs in the CNS is an important tool for studying most fruitful hypotheses regarding the mechanisms of CNS disorders. Studies of the effects of a variety of agonists and antagonists on receptor of GABA, dopamine, serotonin, glutamine or glycine may lead to new concepts pertaining to the pathophysiology of several diseases including insomnia, anxiety, schizophrenia, depression and epilepsy (Bum *et al.*, 2008; Yadav and Nade, 2008; Garba and Yaro, 2015).

Animal models are used to screen the activity of neurochemical agents on the receptors. For example schizophrenia involved mainly the neurotransmitters dopamine and glutamine hence antagonism of dopamine D₂ receptors is a common feature of antipsychotic drugs (Lipska and Weinberger, 2000) while anxiety involves GABA neurotransmitters and enhancing of GABA_A receptors is a common nature or property of anxiolytic drugs. Animal models also are used to screen disorders such as insomnia, anxiety, depression and epilepsy among others. The common animal models used in the screening of neurological and neuropsychiatric disorders include diazepam-induced sleep, hole-board test, beam walk test, elevated plus-maze test, staircase test, open field

test, haloperidol-induced catalepsy, apomorphine-induced stereotypic climbing behaviour, tail suspension, pentylenetetrazole-, picrotoxin- and maximal electroshock-induced convulsion among others. The most convenient animals for initial test (Screening) are usually small rodent such as mice, rats, guinea pigs, rabbits, also small dogs and monkeys are used as well. However, the smaller animals are economical and relatively easy to handle (Ellenberger, 1993).

The common features of most drugs used in the treatment of neuropsychiatric and neurological disorders are inhibition or stimulation of receptors. These receptors include γ -amino butyric acid (GABA), dopamine, serotonin, glutamate, N-methyl-D-aspartate (NMDA), histamine and glycine among others. For example, most anxiolytic drugs act on GABA receptors to enhance GABA mediated inhibition which facilitate the opening of GABA activated chloride channels (Vogel and Vogel, 2002) and sedative drugs act via GABA, histamine or NMDA receptor. However, abnormalities in GABA system have been observed in neurological and psychiatric disease such as insomnia, anxiety, pain attack and epilepsy (Johnston, 2005). Antipsychotic drugs used in the treatment of schizophrenia act on dopamine D₂-receptor, while stimulant, such as strychnine act on glycine receptors (Rang *et al.*, 1998); serotonin on the other hand is involved in the regulation of several aspects of behaviour including sleep, pain perception, depression, sexual activity and aggressiveness (Charles and Robert, 1997); some of the most important antidepressant agents are believed to prevent the reuptake of serotonin.

2.2.1 Benzodiazepines

Benzodiazepines (BZDs) include alprazolam, clonazepam, diazepam, oxazepam among others, are effective in prevention of pentylenetetrazole-induced seizure and they are the most commonly used group of anxiolytics and sedative-hypnotic drugs (Rang *et al.*, 1995). GABA was implicated in the mechanism of benzodiazepine and related CNS drugs (Johnston, 2005). BZDs binding to the specific site of GABA-receptors and these receptors composed of alpha (α), beta (β) and gamma (γ) subunits (Howland and Mycek, 2006). Behavioural analysis of mice with various mutations of the GABA_A receptor subunit indicates that α_1 -receptor mediate the anticonvulsant, sedative-hypnotic and addiction without anxiolytic effect, α_2 -receptor mediate the anxiolytic effect, α_2 , α_3 and α_5 -receptors mediate muscle relaxant while α_1 and α_5 -receptors mediate the amnesic effect (Tan *et al.*, 2011). GABA_A and GABA_B-receptors were identified in mammal, but GABA_A-receptors are found to couple with GABA and induced the shifting of chloride ions (Cl^-), resulting in hyperpolarization of the neuron (Charles and Robert, 1997). However, BZDs enhance GABA-induced increases in Cl^- conductance by increasing the frequency of Cl^- channel opening and this is probably involved in the CNS activity (Page *et al.*, 2002).

Benzodiazepines possess a greater advantage over other CNS depressant drugs such as barbiturates due to its margin of safety which involved the separation between the dose that produces sleep and dose that produces death (Charles and Robert, 1997). The development of dependence is common after regular use of BZDs, even in therapeutic doses for short periods. The most common adverse effects of the BZDs are drowsiness, sedation, muscle weakness and ataxia. Other less frequent side effects include

anterograde amnesia, blurred vision, diplopia, nystagmus, urticaria and rash, hiccups, changes in salivation and bizarre behaviour (Gregory and Strong, 2005).

2.2.2 Barbiturates

Barbiturate is a class of compound having marked CNS depressant properties (Charles and Robert, 1997). Phenobarbitone, pentobarbitone and amobarbitone are among the members of barbiturate and they are sedative-hypnotic drugs which also bind to receptors associated with the GABA chloride ionophore but these drugs potentiate GABAergic inhibition by increasing the duration of Cl⁻ channel opening induced by GABA (Page *et al.*, 2002). The use of barbiturates as sedative-hypnotic agents decreased primarily because of their high addiction liability and the danger of acute lethality and has been replaced with safer benzodiazepine (Charles and Robert, 1997).

2.2.3 Butyrophenones

Antipsychotic drugs include the older phenothiazine and butyrophenones as well as newer atypical drugs (Katzung *et al.*, 2012) and they have affinity for dopamine receptors. Butyrophenones (e.g. bromperidol, haloperidol and droperidol) possess antiemetic properties due to their central dopaminergic blockade. Droperidol is related to haloperidol and at antiemetic doses, it is extremely sedative (Rang *et al.*, 1995). However, haloperidol is widely used antipsychotic drug with a full antagonist at postsynaptic striatal D₂ and D₁ receptors (Bennett and Brown, 2008) and effective in the management of schizophrenia.

2.2.4 Phenothiazines

Phenothiazines are among the older antipsychotic drugs which include prochlorperazine, promethazine thiethylperazine and they are potent antiemetic and sedatives. The antiemetic activities of phenothiazine are mediated through inhibition of dopamine and muscarinic receptors while sedative properties due to their antihistamine activity (Rang *et al.*, 1995).

2.2.5 Hydantoin

Phenytoin (diphenylhydantoin) is the most important member of hydantoin group and structurally related to the barbiturates (Rang *et al.*, 2016). Phenytoin is a valuable agent for the treatment of generalized tonic-clonic and partial seizures (Harvey and Champe, 2006). It is highly effective in reducing the intensity and duration of electrically induced convulsions in mice, although ineffective against pentylene-tetrazole-induced convulsions (Rang *et al.*, 1995). Phenytoin affects membrane excitability by action on voltage-dependent sodium channels, which carry the inward membrane current necessary for the generation of an action potential (Charles and Robert, 1997). Phenytoin not only causes use-dependent block of sodium channels but also affects other aspects of membrane function, including calcium channels and post-tetanic potentiation, as well as intracellular protein phosphorylation by calmodulin-activated kinases, which could also interfere with membrane excitability and synaptic function (Rang *et al.*, 2007).

2.2.6 Other drugs

Drugs such as Flumazenil, pentylenetetrazole, bicuculline and picrotoxin are GABA_A receptor antagonists whereas phencyclidine, ketamine and dizocilpine (MK801) are N-methyl-D-aspartate (NMDA) receptor antagonists (Rang *et al.*, 2016) and blocking of glutamate-activated NMDA receptor channel by ketamine may result in CNS depression or anticonvulsant activity (Manocha *et al.*, 2001). Flumazenil antagonised the effect of BZDs on GABA_A receptor (Rang *et al.*, 2016) and possesses anxiogenic and proconvulsant activity. Bicuculline is a competitive antagonist to GABA_A while picrotoxin is a selective non-competitive antagonist of GABA at GABA_A receptor which has been widely implicated in epilepsy (Rang *et al.*, 1998). However, bicuculline and picrotoxin causes generalized convulsions (Katzung *et al.*, 2012). Pentylenetetrazole is a selective antagonist to GABA_A receptors by blocking the inhibitory effects of GABA (Rang *et al.*, 1998).

Apomorphine is a derivative of morphine having structural similarities to dopamine and a full agonist at D₁ and D₂ receptors (Bennett and Brown, 2008). However, it is a short-acting central and peripheral dopamine receptor agonist. Apomorphine has been used with limited success in ameliorating the symptoms of parkinson's disease and to induce emesis (Charles and Robert, 1997). Apomorphine induced emesis by acting directly on the chemoreceptor trigger zone (CTZ) or direct stimulation of postsynaptic striatal and mesolimbic dopamine receptors to induce stereotypic climbing behaviour (Costall *et al.*, 1978).

Imipramine is an effective drug for the treatment of depression and the most widely used tricyclic antidepressant in the management of panic disorder (Charles and Robert, 1997). Imipramine has been used to control bed wetting in children by causing contraction of the internal sphincter of the bladder (Howland and Mycek, 2006). Tricyclic antidepressants block noradrenaline and serotonin uptake into the neurons. Imipramine and amitriptyline are the prototype drugs as mixed noradrenaline and serotonin uptake inhibitors (Katzung *et al.*, 2012). Blockade of 5-hydroxytryptamine (5-HT) re-uptake contributes to orthostatic hypotension due to the effects on serotonergic neurons in the brainstem vasomotor centre. Imipramine, amitriptyline and nortriptyline have about equal activity at α_1 -adrenergic receptors but imipramine cause significantly more orthostatic hypotension than nortriptyline, this is because imipramine block 5-HT reuptake while nortriptyline block 5-HT receptor (Richelson, 1982).

Valproic acid became the major antiepileptic drug against several seizures. It alone or in combination are used in the treatment of generalized tonic-clonic epilepsy and for partial seizures associated with adverse effect (Katzung *et al.*, 2012). The most serious adverse effect associated with valproic acid is fatal hepatic failure. Valproates appeared to share the ability to block T-type low-voltage-activated calcium channels. T-type channel activity is important in determining the rhythmic discharge of thalamic neurons associated with absence seizure (Khosravani *et al.*, 2004). Valproic acid is not a CNS-depressant, but in combination with other CNS-depressant agents, it may lead to increased depression (Charles and Robert, 1997). Valproate blocks voltage-dependent sodium channels at therapeutically relevant concentration which caused an increase in brain GABA.

2.3 Medicinal Plants with Central Nervous System Activity

Plants are used in traditional medicine and served as source of raw materials for developing new pharmaceutical products (Bagchi *et al.*, 2015). Medicinal plants have both positive and negative values, especially their usage as food and therapeutic applications on one hand. Herbal medicine continues to influence the medicines of today and up to 25 % of all prescription drugs in the United States have at least one active ingredient that comes from plant extracts or synthesized plant compounds (Madhuri and Pandey, 2009). Herbs have been used for medicinal purposes for the treatment of various diseases since prehistoric era. However, since ancient time, man was able to know that, some fruits, stem and leaves of many plants can be used to cure some diseases including treatment of wounds as done by our rural folks (Klein, 1979). Drugs that are obtained from plants were observed to be less toxic and free from side effects than synthetic drugs (Momin, 1987). According to the World Health Organization (WHO), 80 % of the earth's population are estimated to use some form of herbal medicine in their health care (Madhuri and Pandey, 2009). However, people are looking for alternative remedies for the treatment of neurological and psychiatric disorders (Pandy *et al.*, 2012). A number of African medicinal plants have been shown to possess active principles which make them beneficial in the management of neurological and neuropsychiatric disorders. These include *Diospyros mespiliformis* (Adzu *et al.*, 2002), *Dalbergia saxatilis* (Yemitan and Adeyemi, 2013), *Holoptelea integrifolia* and *Tabebuia rosea* (Kavyasree *et al.*, 2013), *Capparis zeylanica* (Banoth and Thaakur, 2014), *Ficus platyphylla* (Chindo *et al.*, 2014) among others. Herbal medicine or phytomedicine are assumed to play a greater role in the management of

neurological and neuropsychiatric disorders and enrich the economic condition of people (Gureje *et al.*, 2006).

2.4 The Plant *Tapinanthus globiferus*

Tapinanthus globiferus is the most common mistletoe that grows on branches of *Vitellaria paradoxa* tree (host) in West Africa and it's a major cause of *Vitellaria paradoxa* mortality in the Northern limits of the Savannah (Watson, 2001). *T. globiferus* is locally known as mistletoe (English), *Kauchin kadanya* (Hausa), *Eme-emi afomo* (Yoruba), and *Osisi/Okwuma osa* (Igbo) in Nigeria and belongs to the family of Loranthaceae (Burkill, 2000). *T. globiferus* is a semi-parasite with glabrous pendulous stems up to 1.2 m long with presumable roots that mostly grow or attache on the branches of a large number of tree species such as *Vitellaria paradoxa*, *Kola*, *Citrus*, *Combretum*, *Acacia*, *Aloe* and *Terminalia* as host tree (Waterberg *et al.*, 1989). It's semi-parasitic because most mistletoe photosynthesizes, although they may obtained up to 60% of their carbohydrates as well as the supply of water and minerals from the host (Watson, 2001). *T. globiferus* has been reported to contained alkaloids, flavonoids, saponins, tannins and cardiac glycosides (Bassey, 2012) and known to have various pharmacological activities on man and animals. Several researches on *T. globiferus* were reported to possess a number of therapeutic uses for managing a wide range of diseases such as diabetes mellitus and hypertension (Akanji *et al.*, 2009; Ogbonnia *et al.*, 2012), oxidative stress and inflammation (Adekunle *et al.*, 2012; Adekunle, 2012) and trypanosomiasis (Abedo *et al.*, 2013a). Comparative study on trypanocidal activity and phytochemical screening of *T. globiferus* and *Gongronema latifolium* were also reported (Abedo *et al.*, 2013b).

2.4.1 Ethnomedicinal uses

Tapinanthus globiferus is used locally for the treatment of various disease including diabetes and stroke (Ogbonnia *et al.*, 2012). In Sudan, *T. globiferus* is used to make a lotion for the treatment of itching (Burkill, 2000). In Saudi Arabia, fresh stems of *T. globiferus* (local name, *Hadhal*) is given orally to all types of livestock for the treatment of fever and removal of placenta after parturition (Sher and Alyemeni, 2011). *T. globiferus* have been used in traditional medicine in the management of hypertension, epilepsy, pain relief, tinnitus and trypanosomiasis (Abedo *et al.*, 2013a). Fresh leaves of *T. globiferus* crushed in cold water served as remedies for tumour in South-western Ethiopia (Yineger and Yewhalaw, 2007). A mixture of one handful each of fresh leaves of *T. globiferus* and root bark of *Boswellia odorata* macerated in 5L of local beer and taken orally for two weeks daily are used to treat Syphilis in the Ebolowa region of Cameroon (Noumi and Eloumou, 2011). *T. globiferus* and *Treculia africana* mixture were observed to have good postprandial sugar lowering effect (Ogbonnia *et al.*, 2012). In Nigeria, the Hausa and Fulani tribes of Northern Nigeria use *T. globiferus* in the treatment of insomnia, convulsion, cancers and inflammations (Abubakar *et al.*, 2007). In Northern Nigeria, *T. globiferus* is used in traditional medicine to treat inflammations, malaria, bacterial infections, ulcer, headaches, insomnia, anxiety, diabetes mellitus, stroke, stomach problems, as well as convulsions (personal communication).

2.4.2 Mistletoe plant

Generally, Mistletoe species are used as an all-rounder plant to treat hypertension, diabetes, cancer, inflammations, cholera, fibroids, migraine, wound infections, and gastrointestinal tract (Ibatomi *et al.*, 1994; Deeni and Sadiq, 2002). In West Africa,

mistletoes are found on many tree crops of economic importance including the Shea butter tree (*Vitellaria Gaertn. F.*), the neem tree (*Azadirachta indica L.*), Citrus species, especially sweet orange (*Citrus sinensis L.*), grape (*Citrus paradise L.*), cocoa (*Theobroma cacao L*) and rubber (*Hevea brasiliensis Muell Arg*) (Adesina *et al.*, 2013). African mistletoes are commonly used in complementary medicine as anticancer, anti-diabetes, bacteriostatic and antihypertensive agents (Orji *et al.*, 2013). Some pharmacological studies on the various mistletoes extract revealed that, the extract have hypotensive, hypoglycaemic, antilipidaemic, anti-oxidative, anti-inflammatory and antimicrobial properties (Jadhav *et al.*, 2010; Ogonnia, 2012; Samba *et al.*, 2015). Several research on many mistletoe reported to possess a number of therapeutic uses for managing a wide range of diseases such as bacterial infection, diabetes mellitus, stroke, headaches, stomach problems, heart palpitations, high blood pressure and breathing difficulties (Osadele and Ukwueze, 2004; Karakas *et al.*, 2008; Basse, 2012). *Loranthus micranthus* (mistletoe) has also been used in the treatment of epilepsy, infertility, menopausal syndrome and rheumatism (Osadele and Ukwueze, 2004).

2.4.3 *Vitellaria paradoxa* (*Tapinanthus globiferus* host plant)

Most mistletoe species have evolved to mimic the foliage of their host tree and also their efficacy depending on the host tree. *Vitellaria paradoxa* commonly called shea butter, belongs to the family of Sapotaceae and has being used in traditional medicine in the treatment of epilepsy and diseases related to the brain like agitations, anxiety, dizziness, headaches, insomnia, migraines, pains and schizophrenia (Burkill, 1995). Studies on the host plant (*Vitellaria paradoxa*) have shown that, the plant has antimycotic, antimicrobial and antifungal activities (Obafemi *et al.*, 2006; Ahmed and

Sani, 2013). Shea butter obtained from the host nuts are traditionally used in medicine, particularly for the preparation of ointments, which are used for the treatment of inflammation, rashes in children, dermatitis, irritation, ulcer, rheumatism, fungal and microbial infections (Marantz and Wiesmann, 2003). However, the leaf decoctions are used for stomach ache and headache, and root powder are taken orally for the treatment of fever with jaundice (Obafemi *et al.*, 2006) while bark decoction are used in a bath to facilitate child birth and drunk to encourage lactation after delivery (Burkill, 1995).



Plate I: *Tapinanthus globiferus* Mistletoe (insert) on *Vitellaria paradoxa* Tree

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Preparation of the Plant Materials

Fresh *Tapinanthus globiferus* (whole mistletoe) grown on *Vitellaria paradoxa* was collected in June, 2015 from Huguma village of Takai Local Government Area, Kano State, Nigeria. *T. globiferus* was identified and authenticated at the Herbarium unit, Department of Botany, Ahmadu Bello University Zaria, Nigeria by comparing with existing voucher specimen number 1052. The plant material was dried under shade until constant weight, crushed and pounded into fine powder. The extraction was conducted with 3,500 g of powdered *T. globiferus* using soxhlet extractor and corresponding output was presented in the results. Ethanol (75% in water) was used as solvent for the extraction and the extract was concentrated on a water bath at temperature of 60°C.

3.2 Fractionation of Ethanol Extract of *T. globiferus*

Fractionation was conducted according to the method described by Ode *et al.* (2011). It was carried out by dissolving 150 g extract in 250 mL water then filtered to have residue and filtrate portions and partitioned with hexane, chloroform, ethylacetate and butanol using separating funnel (Figure 3.1). The fractions were dried using rotary evaporator.

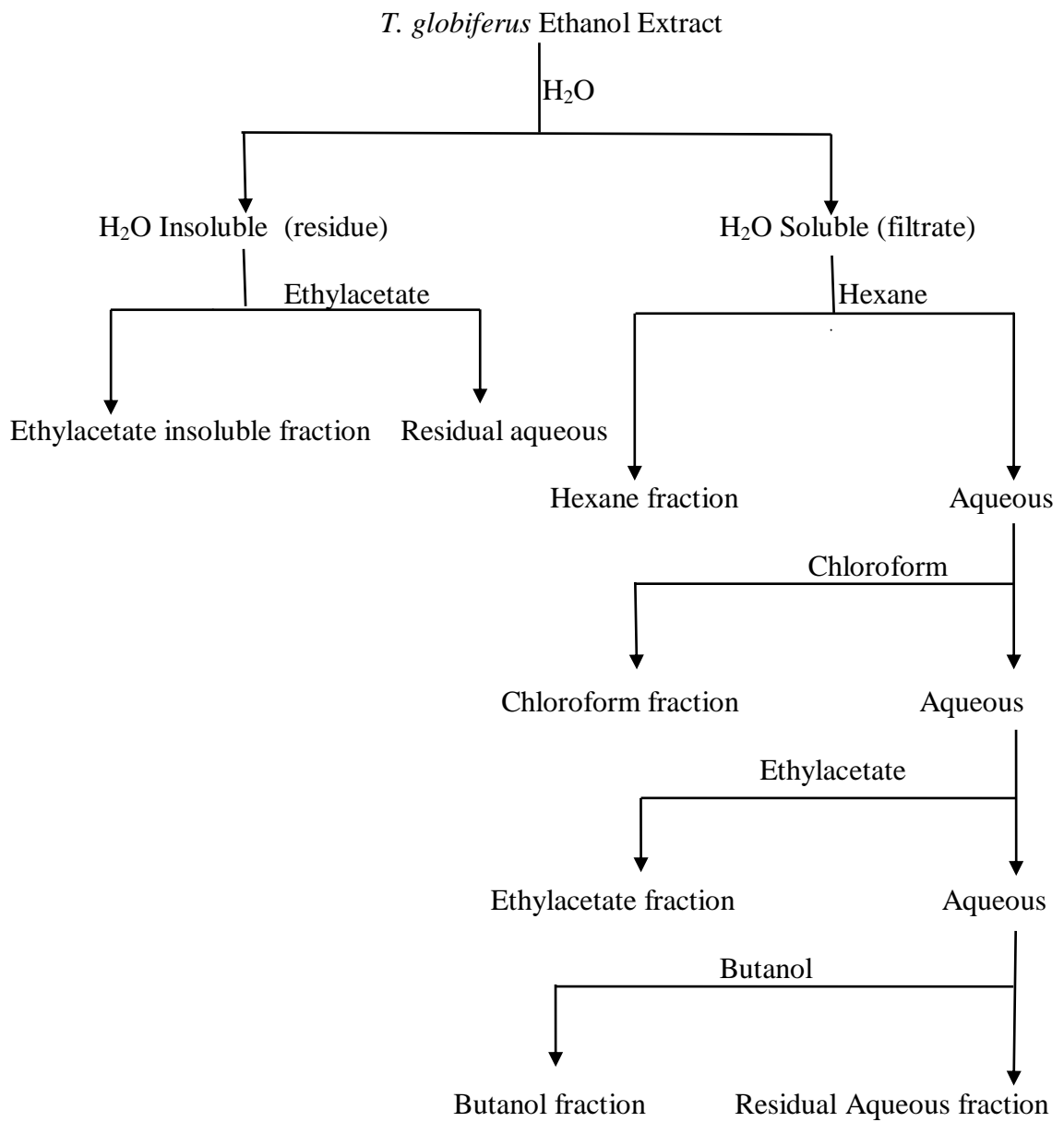


Figure 3.1: Fractionation of Ethanol Extract of *T. globiferus* (Ode *et al.*, 2011)

3.3 Animals

Wistar Rats (150 – 180 g) age between 10 – 15 weeks and Swiss Albino Mice (20 – 25 g) age between 6 – 8 weeks were obtained from Animal House, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria and kept in a well-ventilated room, fed with pelletized grower's mash (Vital feeds, Plc Jos) and water *ad-libitum* was provided throughout the period of the experiment. Day old cockerels (28 – 30 g) were obtained from the National Animal Production and Research Institute (NAPRI), Shika, Zaria. Mice and rats were kept in different cages according to their sex and were allowed to acclimatise prior to experiment. However, during the experiment they were grouped in a simple ratio and distributed according to their sex and weight.

3.4 Drugs, Chemicals and Equipment

Drugs and chemicals were obtained from reputable scientific suppliers such as F. Hoffmann-La Roche Ltd, Basel, Switzerland (diazepam), Sanofi-synthelabo Ltd-UK (Sodium valproate), Sigma Aldrich Inc. USA (apomorphine, bicuculline, ethanol, imipramine, pentylenetetrazole and strychnine), Sterop-Belgium (haloperidol, phenobarbitone and picrotoxin), Rotexmedica, Tittan-Germany (ketamine), Parker-Davis and Co Ltd Detroit (phenytoin) and Dana Plc Nigeria (Normal saline). Electroconvulsive machine used was Ugobasile (Model No. 7801, VA, Italy).

3.4.1 Preparation of drug solutions

The ethanol extract and fractions of *Tapinanthus globiferus*, apomorphine, imipramine, bicuculline, pentylenetetrazole, phenytoin, picrotoxin, sodium valproate and strychnine were prepared by dissolving the powder in normal saline prior to administration.

Diazepam, haloperidol, ketamine and phenobarbitone were supplied in ampoules and appropriate dilutions were made with normal saline. The drug solutions were usually prepared fresh during the experiment to maintain stability of the drugs.

3.4.2 Routes of drug administration

The ethanol extract and fractions of *T. globiferus*, diazepam, phenobarbitone, phenytoin, imipramine, sodium valproate and haloperidol were administered intraperitoneally while apomorphine, pentylenetetrazole, picrotoxin and strychnine were administered subcutaneously.

3.5 Qualitative Phytochemical Screening

The qualitative phytochemical screening of ethanol extract and fractions of *T. globiferus* for the presence or absence of alkaloids, anthraquinones, cardiac glycosides, coumarins, flavonoids, saponins, steroids, terpenoids and tannins were conducted using standard methods as outline below.

3.5.1 Test for alkaloids

Small portion of the extract was dissolved in 5ml of 1% hydrochloric acid (HCl). The mixture was filtered and the filtrate was divided into 3 equal portions. The portions were treated with few drops of Mayer's, Dragendroff's and Wagner's reagent respectively. The buff, orange-red and dark-brown precipitate respectively, indicated the presence of respective alkaloids (Harbone, 1989).

3.5.2 Test for anthraquinones (Borntrager's Test)

3 g of the extract was shaken with 10 ml benzene. This was filtered and 5 ml of 10% ammonia solution was added to the filtrate. The mixture was shaken and the presence of a red colour in the ammonia (lower) phase indicated the presence of free hydroxy anthraquinones (Harbone, 1973).

3.5.3 Test for cardiac glycosides (Keller- Kiliani's Test)

0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing 1 ml of ferric chloride solution, followed by addition of few drops of concentrated sulphuric acid (H₂SO₄). A brown ring obtained at the interface indicated the presence of a desoxy sugar characteristic of cardenolides. Appearance of greenish-blue ring within a few minutes indicated the presence of cardiac glycosides (Trease and Evans, 1983).

3.5.4 Test for coumarins (Feigl's reaction)

About 2 g of the extract was dissolved in distilled water. The solution was divided into 2 equal portions. The first portion was served as reference while second portion was alkaline with addition of 0.5 ml ammonia solution (10%). The occurrence of an intense fluorescence under U.V light indicated the presence of coumarins (Evans, 2002).

3.5.5 Test for flavonoids (Shinoda's Test)

A small portion of the extract was dissolved in ethanol and warmed. The solution was filtered and a piece of magnesium turnings was added to the filtrate, followed by addition of few drops of concentrated hydrochloric acid (HCl). The presence of bubble and pink colour indicated the presence of flavonoids (Venkataram, 1962).

3.5.6 Test for saponins (Frothing Test)

1 g of the extract was shaken vigorously with distilled water and was allowed to stand for 10 min. Persistent froth indicated the presence of saponins (Sofowora, 1984).

Blood haemolysis test: In order to remove false-positive results, the blood haemolysis test was performed on the extract. A small portion of extract that gave positive frothing was shaken with about 2 ml of distilled water. About 1ml of blood sample in saline was added to a portion of the sample and set aside. The blood solution was observed to haemolyse and indicated the presence of saponins (Harbone, 1973).

3.5.7 Test for tannins

2 g of the extract was dissolved and boiled in 10 ml of water, cooled and filtered. The filtrate was treated with few drops of 1% Iron (III) chloride solution. The blue-black, green or blue-green precipitate indicated the presence of tannins (Trease and Evans, 1989).

3.5.8 Test for terpenoids and steroids (Liebermann-Burchard Test)

1 g of the extract was dissolved in 5 ml of chloroform (CHCl_3). The solution was shaken vigorously and then filtered. 1 ml of acetic anhydride was added to the filtrate, followed by addition of few drops of concentrated H_2SO_4 down the side of the test tube. A brown ring at interface and greenish at upper layer indicated the presence of steroids and change from pink to violet colour indicated the presence of terpenoids (Trease and Evans, 1983).

3.6 Acute Toxicity Studies

Median lethal dose (LD₅₀) determination was conducted using the method of Lorke (1983) in which 12 experimental Wistar rats were used. This method involved two phases; in phase I, 9 rats were randomly divided into 3 groups of 3 each. The three groups were treated with the ethanol extract of *T. globiferus* at doses of 10, 100, and 1000 mg/kg intraperitoneally. Animals were observed for signs and symptoms of toxicity including death within 24 hours. The doses used in phase II were guided by phase I results. In phase II, 3 rats were grouped into 3 of one rat each and then treated with the extract at doses of 1,600, 2,900 and 5,000 mg/kg body weight intraperitoneally. Rats were observed for 24 hours and the final LD₅₀ value was calculated as:

$$LD_{50} = \sqrt{X * Y}$$

Where: X = maximum dose that did not killed

Y = minimum dose that killed

The same procedure conducted in the Wistar rats was done in the Swiss Albino mice and also using the different routes of administration to determine LD₅₀ of the ethanol extract and fractions of *T. globiferus*.

3.7 Sub-acute Toxicity Studies

The method of Hodge and Sterner (1943) was used. Twenty four (24) Wistar rats of either sex were used for this study. The rats were divided into four (4) groups of six (6) each and they were divided in a simple ratio and distributed according to their sex and weight across the groups. The first group served as control and were treated with

distilled water (1 ml/kg body weight), while the second, third and fourth groups were treated with 87.5, 175 and 350 mg/kg of the ethanol extract of *T. globiferus* orally respectively on daily basis for twenty eight (28) days, after which rats were sacrificed on the 29th day and organs (liver, kidney and spleen) were harvested for histopathological analysis. Blood samples were collected for biochemistry and haematological evaluations. Body weight, food, water consumption and local injury were monitored daily. Body weight was recorded on days 0 (base line), 7, 14, 21 and 28 of treatments.

3.7.1 Haematological study

Blood samples were collected through cardiac puncture into heparin containing tubes and analysed for the haematological parameters such as packed cell volume (PCV), haemoglobin (Hb), red blood cells (RBC), white blood cells (WBC) and WBC differentials such as neutrophils (Neut), lymphocytes (Lymp), monocytes (Mono), eosinophils (Eosi) and basophils (Baso) using SYSMEX SF-KX-21N Automated Haematology analyser (Sysmex Corporation, Kobe, Japan) (Stiene-Martin *et. al.*, 1998).

3.7.2 Biochemistry study

Blood was collected by cardiac puncture from rats for test of liver and renal function indices. Serum alkaline phosphatase (ALP) was estimated using method of Rec (1987) as described by Randox laboratories, United Kingdom using Randox kits while those of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated using Randox kits according to the method of Reitman and Frankel (1957). Bilirubin (total and conjugated) was estimated using the method of Jendrassik and Grof (1938)

while total protein by using method of Tietz (1995). Serum urea was determined using the method of Weatherburn (1967). Urea in serum is hydrolysed to ammonia in the presence of urease. The ammonia is then measured using spectrophotometer (Model Spectrum Lab. 52s, New Life Medical Instrument England) at 546 nm by Berthelot's reaction and serum creatinine was estimated using method of Bartels and Bohmer (1972). Serum creatinine reacts with picric acid to produce a coloured compound creatinine alkaline picrate which is photometrically read at 492 nm. Serum sodium (Na^+) and potassium (K^+) were estimated using the method of Henry (1974) and the absorbance were measured against the reagent blank at 680 and 500 nm respectively. Serum chloride (Cl^-) and bicarbonate (HCO_3^-) were determined according to the method of Schales and Schales (1941) and Forrester *et al.*, (1976) and they were estimated photometrically at 480 and 340 nm respectively.

3.7.3 Histopathological study

Method of Avwioro (2010) was used. Wistar rats were sacrificed on the 29th day following chloroform anaesthesia. The organs (liver, kidney and spleen) were harvested and preserved in 10% formalin solution. These organs were fixed with 10% normal saline, dehydrated with alcohol, cleared with toluene, infix-treated with molten paraffin wax and embedded in paraffin wax. The microtome sections of the tissues were stained with haematoxylin and eosin staining techniques. Slides were prepared and observed using a standard light microscope (magnification x100).

3.8 Neuro-behavioural Studies

3.8.1 Diazepam-induced sleep in mice

The method of Rakotonirina *et al.* (2001) was used. Twenty-four mice of either sex were divided into four groups of six each. The first group served as control and was treated with normal saline (10 ml/kg, body weight, *i.p.*). The second, third and fourth groups were treated with graded doses of ethanol extract of *T. globiferus* (87.5, 175 and 350 mg/kg, body weight *i.p.* respectively). Thirty minutes after the *i.p.* treatment, mice in all groups received 25 mg/kg body weight of diazepam *i.p.* Mice were placed individually in cages. The onset and duration of sleep were determined for each mouse. The time interval between diazepam administration and loss of righting reflex was considered as onset of sleep while time from the loss to recovery of righting reflex as the duration of sleep.

Diazepam-induced sleep in mice was repeated with the fractions of ethylacetate at doses of 75, 150 and 300 mg/kg body weight *i.p.*, insoluble ethylacetate at doses of 50, 100 and 200 mg/kg body weight *i.p.* while butanol and residual aqueous fractions at the same doses of 250, 500 and 1,000 mg/kg body weight *i.p.* However, yield of the chloroform and hexane fractions were negligible.

3.8.2 Ketamine-induced sleep in mice

Ketamine-induced sleep in mice was used for interactive study and method of Mimura *et al.* (1990) was adopted. Twenty-four mice of either sex were divided into four groups of six each. The first group served as control and was treated with normal saline (10 ml/kg, body weight, *i.p.*). The second, third and fourth groups were treated with graded

doses of ethylacetate fraction (75, 150 and 300 mg/kg, body weight *i.p.* respectively). Thirty minutes after *i.p.* pre-treatment, mice in all groups received 100 mg/kg body weight of ketamine. Mice were placed individually in cages. The time interval between ketamine administration and loss of righting reflex was considered as onset of sleep while the time from the loss to regaining of righting reflex as the duration of sleep.

3.8.3 Hole board test for exploratory behaviour in mice

The method described by File (1973) was adopted for this study. Hole-board is a white painted wooden board (60 x 30 cm) with 16 evenly spaced holes (1 cm diameter x 2 cm depth). Thirty mice were divided into five groups of six each. Mice in the first and second groups received 10 ml/kg normal saline and 2 mg/kg diazepam *i.p.* respectively while mice in the third, fourth and fifth groups received 87.5, 175 and 350 mg/kg, body weight *i.p.* of the ethanol extract of *T. globiferus* respectively. Thirty minutes post-treatment, each mouse was placed at a corner of the hole-board and the number of head dips into the hole during 5 minutes was recorded. Head dip was considered when mouse completely dipped head into the hole to the level of the eyes.

Hole-board test in mice was repeated with the fractions of ethylacetate at doses of 75, 150 and 300 mg/kg body weight *i.p.* and butanol at doses of 250, 500 and 1,000 mg/kg body weight *i.p.* However, insoluble ethylacetate and residual aqueous fractions were neglected.

Hole-board test method was adopted for interactive study. Thirty-six mice were divided into six groups of six each. Mice in group I, II and III were treated with 10 ml/kg

normal saline, 2 mg/kg diazepam and 300 mg/kg ethylacetate fraction respectively only. Thirty minutes after *i.p.* treatment, mice were placed individually at a corner of the hole-board and the number of head dips into the hole during 5 minutes was counted and recorded. Mice in group IV, V and VI pre-treated with 5 mg/kg *i.p.*, body weight of bicuculline, 15 minutes later group V was post-treated with 2 mg/kg *i.p.* diazepam and group VI with 300 mg/kg *i.p.* ethylacetate fraction. Thirty minutes post-treatment, each mouse was placed at a corner of the hole-board and the number of head dips into the hole during 5 minutes was recorded.

3.8.4 Beam walk assay in mice for motor coordination

The beam walking assay was carried out according to the method of Stanley *et al.* (2005). The beam was made of wood (8 mm in diameter and 60 cm long) elevated 30 cm above the bench by metal supports. Mice were trained to walk from a start platform along a ruler (80 cm long and 3 cm wide) elevated 30 cm above the bench by metal supports to a goal box. Three trials were made for each mouse, such that the mice tested would be aware that there was a goal box that could be reached. Thirty trained mice were divided into five groups of six mice each. The first group received 10 ml/kg body weight normal saline *i.p.* while second group received Diazepam (1 mg/kg body weight, *i.p.*). The third, fourth and fifth groups received ethanol extract of *T. globiferus* at doses of 87.5, 175 and 350 mg/kg, body weight *i.p.* respectively. Thirty minutes post-treatment, each mouse was placed on the beam at one end and allowed to walk to the goal box. Mice that fell were returned to the position they fell from. Each mouse was allowed to spend a maximum of 60 sec on the beam. The number of falls, foot slips (one

or both hind limb slipped from the beam) and time spent to reach the goal box were recorded.

Beam walking assay was repeated with the fractions of ethylacetate at doses of 75, 150 and 300 mg/kg body weight *i.p.* and butanol at doses of 250, 500 and 1,000 mg/kg body weight *i.p.* but, insoluble ethylacetate and residual aqueous fractions were neglected.

Diazepam-induced sleep, hole-board test and beam walk assay in mice were selected to screen the activity of the fractions based on the activity of ethanol extract of *T. globiferus* in these models. However, only ethylacetate and butanol fractions were observed to have effect in the aforementioned models.

3.8.5 Elevated plus-maze test in mice for anxiolytic behaviour

The method described by Lister (1987) was adopted for this study. Elevated plus-maze (EPM) apparatus consists of 2 open arms (35 x 5 cm) and 2 closed arms (35 x 5 x 20 cm) connected together with a central square (5 x 5 cm). The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Thirty mice were divided into five groups of six each. First and second groups were treated with normal saline (10 ml/kg body weight *i.p.*) and diazepam (0.25 mg/kg body weight *i.p.*) respectively while third, fourth and fifth groups were treated with 87.5, 175 and 350 mg/kg, body weight *i.p.* of the ethanol extract of *T. globiferus*. Thirty minutes after *i.p.* treatment, mice were placed individually into the centre of EPM facing closed arms. Mouse was allowed to spend maximum of 5 minutes in both open and closed arms. The number of entries and time

spent in the open and closed arms was counted and recorded during 5 minutes. An entry into open or closed arm was defined as having all four paws within the arm.

3.8.6 Staircase test in mice for anxiolytic behaviour

This test was conducted according to the method described by Simiand *et al.* (1984). The staircase was made up of wood and consists of five identical steps 2.5 cm high, 10 cm wide and 7.5 cm deep. The staircase was surrounded by walls of transparent Plexiglas, the height of which was constant along the whole length of the staircase. Thirty mice were divided into five groups of six each. First and second groups were treated with normal saline (10 ml/kg body weight *i.p.*) and diazepam (0.25 mg/kg body weight *i.p.*) respectively while third, fourth and fifth groups were treated with 87.5, 175 and 350 mg/kg, body weight *i.p.* of the ethanol extract of *T. globiferus*. Thirty minutes after *i.p.* treatment, mice were placed individually on the floor of the box with its back on the staircase. The number of steps climbed and rear climbed was counted and recorded during 3 minutes, but number of steps descended was not considered. Step is considered to have climbed when the mouse placed all four paws on the step while rearing is when hind legs either on the step or against the wall to sniff air. The box was cleaned after each trial in order to eliminate olfactory cues which might modify the behaviour of the next animal.

3.8.7 Open field test in mice for locomotor activity

The method of Brown *et al.* (1999) was adopted in this study. The arena of the open field consists of 72 x 72 cm wooden box of 36 cm high in which the floor is divided into 16 squares (15 x 15 cm). Thirty mice were divided into five groups of six each. First

and second groups received normal saline (10 ml/kg) and diazepam (1 mg/kg) respectively (*i.p.*) while third, fourth and fifth groups were treated with 87.5, 175 and 350 mg/kg, body weight *i.p.* of the ethanol extract of *T. globiferus*. Thirty minutes after *i.p.* treatment, each mouse was placed at centre of open field and allowed to explore the apparatus for 5 minutes. After the 5 minutes test, mice were returned into their cage and open field was carefully cleaned with 70% ethanol solution after every test. Each mouse was then given score for locomotor activity. The number of line crossed and rearing was counted and recorded for each mouse.

3.8.8 Haloperidol-induced catalepsy in mice

Catalepsy was induced with haloperidol and assessed by means of a standard bar test at 30 minutes interval for 120 minutes. The study was conducted according to Ferre *et al.* (1990) and modified by Salam (2011). Twenty four adult mice were randomly divided into four groups of six each. The first group received 10 ml/kg body weight *i.p.* of normal saline while second, third and fourth groups received ethanol extract of *T. globiferus* at doses of 87.5, 175 and 350 mg/kg, body weight *i.p.* respectively. Thirty minutes after *i.p.* treatment, mice in all groups were treated with 1.0 mg/kg body weight of haloperidol *i.p.* Mice were positioned so that their hindquarters were on the bench and their forelimbs rested on 1 cm diameter horizontal bar that was 4 cm above the bench, this procedure was performed 30 minutes after the administration of haloperidol. Mice were judged to be cataleptic if they maintained this position. The length of time for which the mouse maintained this position was recorded with a stopwatch with a maximum duration of 180 seconds and the end point of catalepsy was considered to occur when both front paws were removed from the bar.

3.8.9 Apomorphine-induced climbing behaviour in mice

Twenty four adult mice were divided into four groups of six each. First group served as control and received normal saline (10 ml/kg body weight *i.p.*). The second, third and fourth groups were treated with graded doses of ethanol extract of *T. globiferus* (87.5, 175 and 350 mg/kg, body weight) *i.p.* respectively. Thirty minutes after *i.p.* treatment, mice in all groups received 3 mg/kg body weight of apomorphine *s.c.* Mice were placed individually in a wire mesh stick cage and the climbing behaviour was scored at 10 minutes interval for a period of 30 minutes. The scoring system used was as follows: 0 = when the four paws are on the floor, 1 = when the two paws are holding the vertical bars and 2 = when the four paws are holding or climbing the vertical bars (Costall *et al.*, 1978).

3.8.10 Tail suspension test in mice for antidepressant screening

The study was conducted according to Steru *et al.* (1985). Adult mice were randomly divided into five groups of six each. Mice in the first and second groups received normal saline (10 ml/kg) and imipramine (4 mg/kg) respectively (*i.p.*). Third, fourth and fifth groups received graded doses of the ethanol extract of *T. globiferus* (87.5, 175 and 350 mg/kg, body weight) *i.p.* respectively. Thirty minutes after *i.p.* treatment, each mouse was suspended by the tail on the edge of a shelf 58 cm above a table top and length of immobility were recorded for a period of 6 minutes after discarding activity in the first 2 minutes during which the mouse tried to escape. Mouse was considered immobile when hung passively and remains motionless.

3.8.11 Pentylenetetrazole-induced convulsion test in mice

The method of Swinyard *et al.* (1989) was adopted. Thirty mice were divided into five groups of six each. First and second groups received normal saline (10 ml/kg) and sodium valproate (200 mg/kg) respectively (*i.p.*). Third, Fourth and fifth groups were treated with graded doses of ethanol extract of *T. globiferus* (87.5, 175 and 350 mg/kg, body weight) *i.p.* respectively. Thirty minutes after *i.p.* treatment, mice in all groups received 75 mg/kg body weight of pentylenetetrazole subcutaneously and observed for 30 minutes for the onset and incidence of seizures. An episode of tonic extension of the hind limbs which persisted for a minimum period of 30 seconds was taken as threshold convulsion. Lack of threshold convulsion during 30 minutes of observation was regarded as protection. The number of mice protected was recorded and the anticonvulsant properties of the extract expressed as percentage protection.

3.8.12 Strychnine-induced convulsion test in mice

According to Porter *et al.* (1984), thirty adult mice of either sex were divided into five groups of six each. Mice in group I served as control and received normal saline (10 ml/kg *i.p.*) while group II received phenobarbitone (30 mg/kg *i.p.*). Groups III, IV and V received 87.5, 175 and 350 mg/kg, *i.p.* body weight of ethanol extract of *T. globiferus* respectively. Thirty minutes after *i.p.* treatment, mice in all groups received 1.2 mg/kg body weight of Strychnine nitrate subcutaneously (*s.c.*) and they were observed for a period of 30 minutes for the onset and incidence of convulsion. Prevention of tonic hind-limb extension within 30 minutes was considered as an indication of anticonvulsant activity of the extract. Seizure was manifested as tonic hind limb

extension and the ability of the extract to prevent the feature or prolong the latency or onset of tonic hind limb extension was taken as an indication of anticonvulsant activity.

3.8.13 Maximal electroshock-induced convulsion test in chicks

For maximal electroshock test (MEST), the method of Swinyard and Kupferberg (1985) was adopted. Fifty day old chicks were divided into five groups of ten each. Group I were given 10 ml/kg *i.p.* normal saline and Group II received 20 mg/kg *i.p.* phenytoin. Groups III, IV and V received ethanol extract of *T. globiferus* at doses of 87.5, 175 and 350 mg/kg *i.p.* respectively. Thirty minutes later, maximal electroshock was delivered to each chick to induce seizure using an Ugobasile electro-convulsive machine (Model No. 7801) connected with corneal electrodes to eye lid of the chick. The parameters used were 80 mA (current), 100 Hz (frequency), 0.8 sec. (shock duration) and 0.6 ms (pulse width). Onset is the time interval between administration of shock and loss of righting reflex. Time interval from the loss to regaining of righting reflex considered as recovery from seizures and was recorded. The episodes of tonic extension of the hind limbs were regarded as full convulsion while lack of tonic extension of the hind limbs was considered as protection.

3.8.14 Picrotoxin-induced convulsion test in mice

The method adopted for this study was as previously described by Rogawski and Porter (1990). Thirty mice were randomly divided into five groups of six each. Mice in first group were pre-treated with normal saline (10 ml/kg body weight) and second group pre-treated with 30 mg/kg body weight phenobarbitone while third, fourth and fifth groups were pre-treated with ethanol extract of *T. globiferus* at doses of 87.5, 175 and

350 mg/kg body weight respectively, all via intraperitoneal route. Thirty minutes later, all mice were treated with 5 mg/kg picrotoxin via subcutaneous route. They were observed for hind limb tonic extension over 30 minutes period. Absence of tonic hind limb extension or prolongation of the latency of hind limb tonic extension was considered as protection.

3.9 Statistical Analysis

Statistical analysis was carried out using SPSS software (version 20). Data were analysed using One Way Analysis of Variance (ANOVA) followed by Dunnett's *post-hoc* test and values of $p \leq 0.05$ were considered statistically significant. Data obtained over time was analysed using repeated measure ANOVA followed by Bonferroni *post-hoc* test. Results were presented as Mean \pm Standard Error of the Mean (Mean \pm SEM) in tables and figures.

CHAPTER FOUR

4.0 RESULTS

4.1 Percentage Yield of Plant Material

Soxhlet extraction of 3,500 g powdered material of *T. globiferus* yielded 348.41 g of extract which is equivalent to 9.96%.

Fractionations of ethanol extract of *T. globiferus* (150 g) yielded fractions of hexane, chloroform, ethylacetate, ethylacetate insoluble, butanol and aqueous residue in which aqueous residue fraction have the highest percentage yield followed by ethylacetate then butanol while chloroform fraction found to be the least (Table 4.1).

4.2 Phytochemical Constituents of Ethanol Extract and Fractions of *Tapinanthus globiferus*

Qualitative phytochemical screening of the ethanol extract of *T. globiferus* revealed the presence of alkaloids, anthraquinones, flavonoids, cardiac glycosides, saponins, steroids, terpenoids and tannins while coumarins are absent (Table 4.2).

Qualitative phytochemical screening of the fractions revealed the presence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, saponins and tannins in the ethylacetate fraction; anthraquinones, cardiac glycosides, flavonoids saponins and tannins in the butanol fraction; alkaloids, cardiac glycosides and saponins in the aqueous residual fraction while steroids and terpenoids were present in both hexane and ethylacetate water insoluble fractions (Table 4.2).

Table 4.1: Percentage Yield of the Fractions Obtained from Ethanol Extract of *Tapinanthus globiferus*

Fractions	Yield (g)	Percentage yield (% w/w)
Hexane	2.98	4.07
Chloroform	0.87	1.19
Ethylacetate	14.49	19.80
Ethylacetate insoluble	6.72	9.18
Butanol	13.17	17.99
Residual aqueous	34.97	47.77

Table 4.2: Phytochemical Constituents of Ethanol Extract and Fractions of
Tapinanthus globiferus

Constituents	TgE	HF	EF	EIF	BF	RF
Alkaloids	+	-	+	-	-	+
Anthraquinone	+	-	+	-	+	-
Cardiac glycoside	+	-	+	-	+	+
Coumarins	-	-	-	-	-	-
Flavonoids	+	-	+	-	+	-
Saponins	+	-	+	-	+	+
Steroids	+	+	-	+	-	-
Tannins	+	-	+	-	+	-
Terpenoids	+	+	-	+	-	-

TgE = *Tapinanthus globiferus* Ethanol Extract, HF = Hexane Fraction, EF = Ethylacetate Fraction, EIF = Ethylacetate Insoluble Fraction, BF = Butanol Fraction, RF = Residual Fraction, + = Presence, - = Absence.

4.3 Toxicity Studies on Ethanol Extract and Fractions of *T. globiferus*

4.3.1 Acute toxicity studies

Signs and symptoms observed in test animals administered with ethanol extract and fractions of *T. globiferus* included decreased locomotor activity and CNS depression.

The median lethal dose (LD₅₀) of ethanol extract of *T. globiferus* after intraperitoneal (*i.p.*) administration was found to be 1,300 mg/kg in mice and 3,800 mg/kg in rats. However, the LD₅₀ of the extract after oral administration were found to be greater than 5,000 mg/kg in both mice and rats (Table 4.3).

The intraperitoneal LD₅₀ of ethylacetate and ethylacetate water insoluble fractions were found to be 1,400 and 1,100 mg/kg respectively while butanol and aqueous residual fractions was found to be 3,800 mg/kg each in mice (Table 4.3).

4.3.2 Sub-acute toxicity studies

*4.3.2.1 Effect of ethanol extract of *T. globiferus* on body weight of rats following twenty eight days daily oral treatment*

The ethanol extract of *T. globiferus* at doses of 87.5, 175 and 350 mg/kg had no significant ($p \geq 0.05$) effect on body weight of rats after twenty eight days daily oral treatment compared to control rats treated with 1 ml/kg normal saline (Table 4.4).

4.3.2.2 *Effect of ethanol extract of T. globiferus on haematological indices in rats*

following twenty eight days daily oral administration

The twenty eight days daily oral treatment of rats with ethanol extract of *T. globiferus* produced significant ($p \leq 0.05$) decrease in the serum levels of packed cell volume, haemoglobin and red blood cell at dose of 350 mg/kg extract and the serum levels of neutrophil and monocyte insignificantly ($p \geq 0.05$) increased at the same dose of 350 mg/kg extract compared to control rats treated with 1 ml/kg normal saline (Table 4.5).

4.3.2.3 *Effect of ethanol extract of T. globiferus on serum biochemical parameters in*

rats following twenty eight days daily oral administration

The ethanol extract of *T. globiferus* showed no significant ($p \geq 0.05$) effect on rats serum concentrations of AST, ALT, ALP and TP at tested doses (87.5, 175 and 350 mg/kg) after 28 days daily oral treatment. However, a significant ($p \leq 0.05$) increase in TB was observed at dose of 350 mg/kg. The extract also produced a significant ($p \leq 0.05$) and dose dependent increase in serum concentration of CB when compared with control (Table 4.6).

A significant ($p \leq 0.05$) decrease in serum concentration of urea, creatinine and sodium were observed at a dose of 350 mg/kg. Conversely, serum concentration of chloride significantly ($p \leq 0.05$) increased at doses of 175 and 350 mg/kg extract compared to control group treated with 1 ml/kg normal saline. However, there was no significant change in serum potassium and biocarbonate concentration at the tested doses (Table 4.7).

Table 4.3: Median Lethal Dose (LD₅₀) of Ethanol Extract and Fractions of *Tapinanthus globiferus*

	Route of administration	Animal	LD₅₀ values (mg/kg)
TgE	<i>i.p.</i>	Mice	1,300.00
TgE	<i>i.p.</i>	Rats	3,800.00
TgE	Oral	Mice	>5,000.00
TgE	Oral.	Rats	>5,000.00
EF	<i>i.p.</i>	Mice	1,400.00
EIF	<i>i.p.</i>	Mice	1,100.00
BF	<i>i.p.</i>	Mice	3,800.00
RF	<i>i.p.</i>	Mice	3,800.00

TgE = *Tapinanthus globiferus* Ethanol Extract, EF = Ethylacetate Fraction, EIF = Ethylacetate Insoluble Fraction, BF = Butanol Fraction, RF = Residual aqueous Fraction, *i.p.* = intraperitoneal.

Table 4.4: Effect of Ethanol Extract of *Tapinanthus globiferus* on Body Weight of Rats Following Twenty Eight Days Daily Oral Administration

Treatment (mg/kg)	Day 0	Day 7	Day 14	Day 21	Day 28
NS 1 ml/kg	174.17 ± 7.12	176.17 ± 7.07	175.67 ± 7.00	177.33 ± 7.02	175.00 ± 6.88
TgE (87.5)	174.17 ± 5.93	174.33 ± 6.30	175.50 ± 6.02	175.50 ± 6.24	175.17 ± 5.99
TgE (175)	174.00 ± 5.14	174.67 ± 5.25	176.67 ± 5.29	177.33 ± 5.51	175.33 ± 5.44
TgE (350)	174.00 ± 6.06	174.17 ± 6.01	174.17 ± 5.56	174.33 ± 5.49	174.83 ± 5.77

No significant difference between control and treated groups, and over time -repeated measure ANOVA followed by Bonferroni *post-hoc* test. n = 6, Data = Mean ± SEM, TgE = *T. globiferus* Ethanol Extract, NS = Normal saline.

Table 4.5: Effect of Ethanol Extract of *Tapinanthus globiferus* on Haematological Indices in Rats after Twenty Eight Days Daily Oral Administration

Treatment (mg/kg)	PCV %	Hb g/L	RBC x 10 ⁹ /L	WBC x 10 ⁹ /L	NEUT x 10 ⁹ /L	LYMP x 10 ⁹ /L	MONO x 10 ⁹ /L	EOSI x 10 ⁹ /L	BASO x 10 ⁹ /L
NS 1 ml/kg	40.83±1.05	13.55±0.32	6.80±0.18	7.73±0.73	9.50±3.83	85.17±4.09	0.00 ± 0.00	2.67 ± 1.02	0.00
TgE (87.5)	42.33±1.17	14.10±0.39	7.08±0.19	8.08±0.86	11.17±5.50	83.67±5.16	0.00 ± 0.00	3.00 ± 1.37	0.00
TgE (175)	41.67±1.12	13.82±0.38	6.92±0.17	9.95±1.32	7.33±1.05	90.17±1.66	0.60 ± 0.60	0.00 ± 0.00	0.00
TgE (350)	36.67±1.15 ^a	12.17±0.39 ^a	6.05±0.18 ^a	7.37±1.02	21.67±5.65	76.83±5.76	0.17 ± 0.17	1.17 ± 1.17	0.00

^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, TgE = *T. globiferus* Ethanol Extract, NS = Normal saline, PCV = Packed cell volume, Hb = Haemoglobin, RBC = Red blood cell, WBC = White blood cell, NEUT = Neutrophil, LYMP = Lymphocyte, MONO = Monocyte, EOSI = Eosinophil, BASO = Basophil.

Table 4.6: Effect of Ethanol Extract of *Tapinanthus globiferus* on Serum Biomarkers in Rats Following Twenty Eight Days Daily Oral Administration

Treatment (mg/kg)	AST (U/L)	ALT (U/L)	ALP (U/L)	TB (µmol/L)	CB (µmol/L)	TP (g/dl)
NS 1 ml/kg	8.80±3.79	11.60±3.20	50.00±9.00	27.79±0.90	17.52±0.08	6.76±0.17
TgE (87.5)	10.00±1.90	12.60±2.11	59.60±7.61	25.79±0.40 ^a	14.82±0.11 ^a	7.04±0.16
TgE (175)	10.40±1.72	16.00±3.44	54.80±8.41	27.16±0.30	15.77±0.14 ^a	7.04±0.19
TgE (350)	12.50±2.26	21.00±4.99	47.67±8.98	35.21±0.29 ^a	16.93±0.11 ^a	6.67±0.20

^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM. TgE = *T. globiferus* Ethanol Extract, NS = Normal Saline, AST = Aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase, TB = Total bilirubin, CB = conjugated bilirubin, TP = Total protein.

Table 4.7: Effect of Ethanol Extract of *Tapinanthus globiferus* on Serum Urea, Creatinine and Electrolytes in Rats Following Twenty Eight Days Daily Oral Administration

Treatment (mg/kg)	Urea (mmol/L)	Creatinine (mmol/L)	Na⁺ (mmol/L)	K⁺ (mmol/L)	Cl⁻ (mmol/L)	HCO₃⁻ (mmol/L)
NS 1 ml/kg	6.50±0.27	85.40±13.52	143.40±1.00	3.74±0.12	104.20±1.36	23.20±1.24
TgE (87.5)	6.30±0.25	84.00±8.24	138.80±1.60	4.10±0.29	104.20±1.46	23.20±1.40
TgE (175)	5.28±0.44 ^a	80.40±8.10	138.40±1.03	4.40±0.31	108.40±0.40 ^a	22.60±0.75
TgE (350)	4.00±0.76 ^a	53.33±8.91 ^a	124.67±1.93 ^a	4.07±0.10	109.17±0.91 ^a	20.00±1.52

^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM, TgE = *T. globiferus* Ethanol Extract, NS = Normal Saline.

4.3.2.4 Effect of ethanol extract of T. globiferus on functional and structural integrity of rat liver following twenty eight days oral daily administration

Histopathological examination of liver section of wistar rats treated with ethanol extract of *T. globiferus* at doses of 175 and 350 mg/kg showed areas of moderate distortion and degeneration of the hepatocytes respectively while liver section of the rats treated with dose of 1 ml/kg normal saline and 87.5 mg/kg extract showed normal hepatic tubular architecture after 28 days oral daily administration (Plate II).

4.3.2.5 Effect of ethanol extract of T. globiferus on functional and structural integrity of rat kidney following twenty eight days oral daily treatment

Histopathological observation of kidney sections of the rats treated with normal saline (1 ml/kg) and ethanol extract of *T. globiferus* at doses of 87.5 and 175 mg/kg showed normal kidney architecture while kidney section of the rat treated with 350 mg/kg dose of extract showed degeneration of Bowman's capsule, tubular degeneration and blood vessel distraction following 28 days oral daily administration (Plate III).

4.3.2.6 Effect of ethanol extract of T. globiferus on functional and structural integrity of rat spleen following twenty eight days oral daily administration

Histopathological evaluation of spleen sections of the rats following 28 days oral daily treatment with 1 ml/kg normal saline and ethanol extract of *T. globiferus* at doses of 87.5 and 175 mg/kg revealed normal spleen architecture while spleen of the rat that received 350 mg/kg of the extract showed area of degeneration in red pulp and necrosis of the epithelial cells (Plate IV).

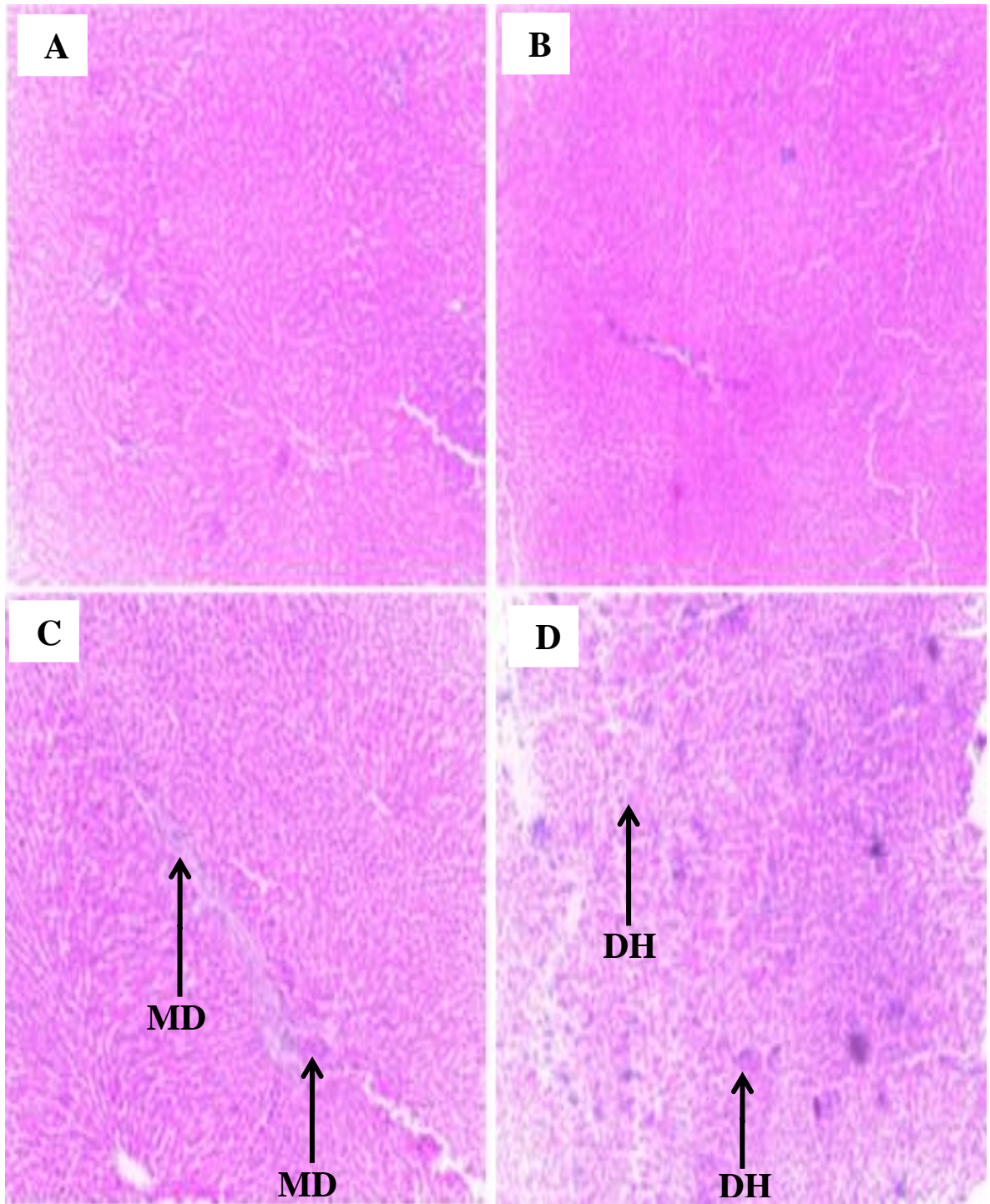


Plate II: Photomicrograph of transverse section of the liver of rats treated with the ethanol extract of *Tapinanthus globiferus*, arrow showing moderate distortion (MD) and degeneration of hepatocytes (DH) following twenty eight days daily oral treatment (H and E stain x100). (A) 1 ml/kg Normal saline; (B) 87.5 mg/kg; (C) 175 mg/kg; (D) 350 mg/kg.

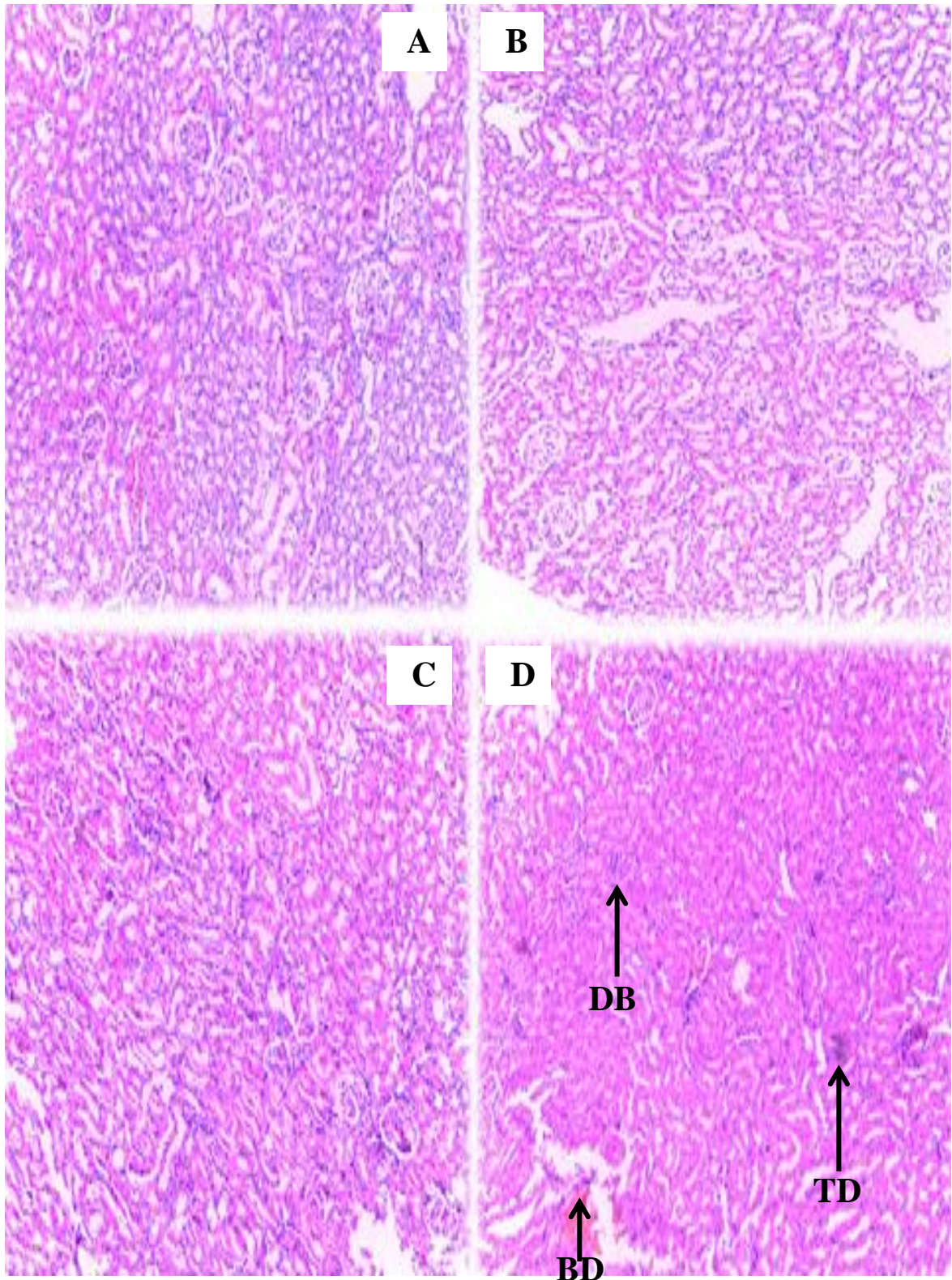


Plate III: Photomicrograph of transverse section of the kidney of rat treated with the ethanol extract of *Tapinanthus globiferus*, arrow showing degeneration of Bowman's capsule (DB), tubular degeneration (TD) and blood vessel distortion (BD) following twenty eight days daily oral treatment (H and E stain x100). (A) 1 ml/kg Normal saline; (B) 87.5 mg/kg; (C) 175 mg/kg; (D) 350 mg/kg.

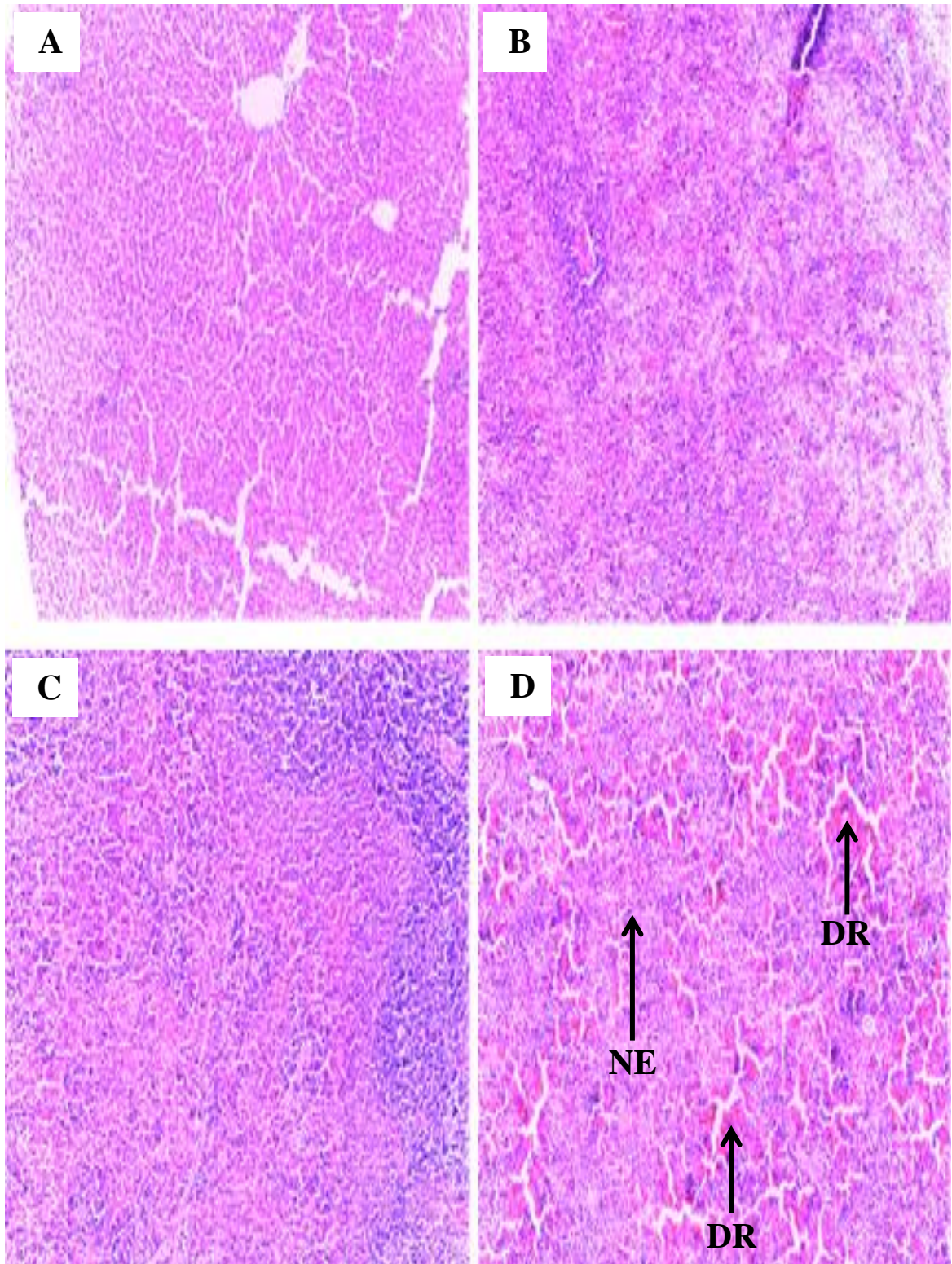


Plate IV: Photomicrograph of transverse section of the spleen of rat treated with the ethanol extract of *Tapinanthus globiferus*, arrow showing degeneration in red pulp (DR) and necrosis of epithelial cell (NE) following twenty eight days daily oral treatment (H and E stain x100). (A) 1 ml/kg Normal saline; (B) 87.5 mg/kg; (C) 175 mg/kg; (D) 350 mg/kg.

4.4 Neuro-behavioural Studies

4.4.1 Effect of ethanol extract and fractions of *T. globiferus* on diazepam-induced sleep in mice

The ethanol extract of *T. globiferus* produced a significant ($p \leq 0.05$) and dose dependent decrease in the onset of sleep in mice at tested doses (87.5, 175 and 350 mg/kg) and increase in the duration of diazepam-induced sleep at doses of 175 and 350 mg/kg compared to control (Fig. 4.1).

The ethylacetate fraction (EF) showed a significant ($p \leq 0.05$) dose-dependent decrease in the onset of sleep in mice at tested doses (75, 150 and 300 mg/kg) and significant ($p \leq 0.05$) increase in the duration of diazepam-induced sleep at doses of 150 and 300 mg/kg compared to control treated with 10 ml/kg normal saline (Fig. 4.2).

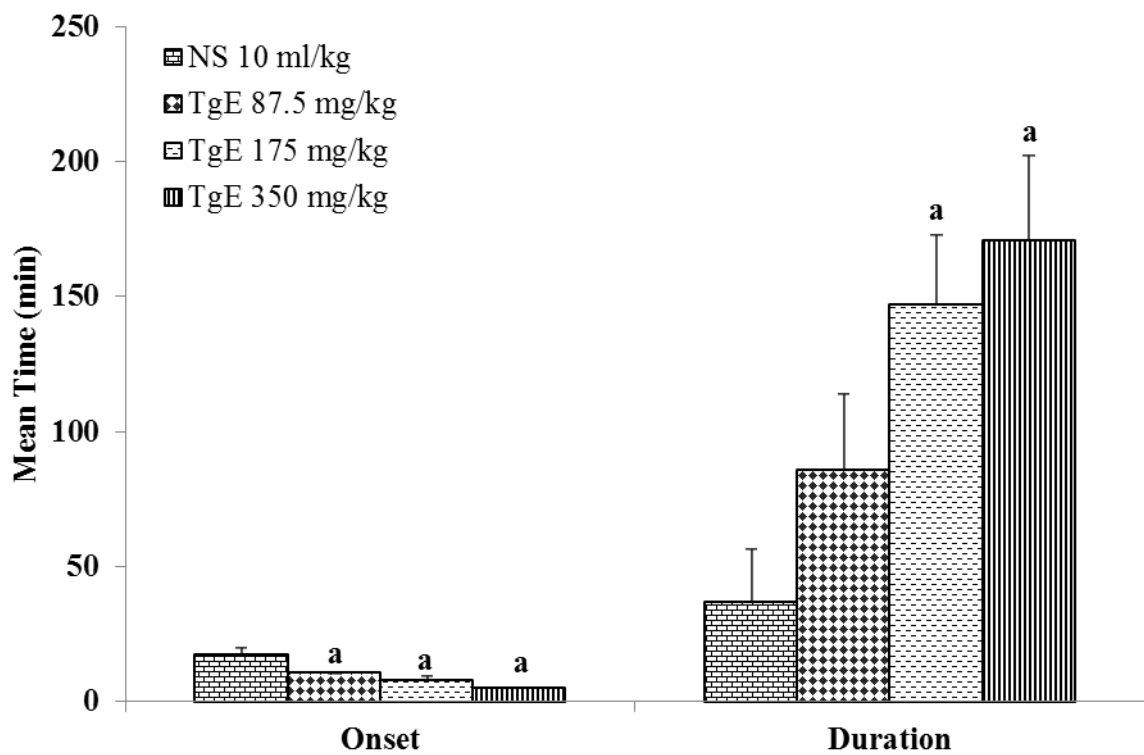
Ethylacetate insoluble fraction (EIF) did not show any significant difference in onset and duration of diazepam-induced sleep in mice at all tested doses (50, 100 and 200 mg/kg) compared to control that received 10 ml/kg of normal saline (Fig. 4.3).

Butanol fraction (BF) produced a significant ($p \leq 0.05$) decrease in the onset and increase in the duration of sleep in mice at doses of 500 and 1,000 mg/kg in diazepam-induced sleep test. However, at dose of 250 mg/kg, BF did not produce any significant difference compared to control (Fig. 4.4).

At doses of 500 and 1,000 mg/kg, residual aqueous fraction (RF) significantly ($p \leq 0.05$) decreased the onset of diazepam-induced sleep in mice. However, the duration of

sleep at tested doses (250, 500 and 1,000 mg/kg) was observed to be insignificant ($p \geq 0.05$) compared to control treated with 10 ml/kg of normal saline (Fig. 4.5).

Effects of EF, EIF, BF and RF were compared and revealed that EF produced significant ($p \leq 0.05$) decrease in the onset and increase in the duration of sleep followed by BF in diazepam-induced sleep test in mice (Fig. 4.6).



SLEEP INDICES

Figure 4.1: Effect of Ethanol Extract of *T. globiferus* on Onset and Duration in Diazepam-induced Sleep in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, Diazepam (25 mg/kg), route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline.

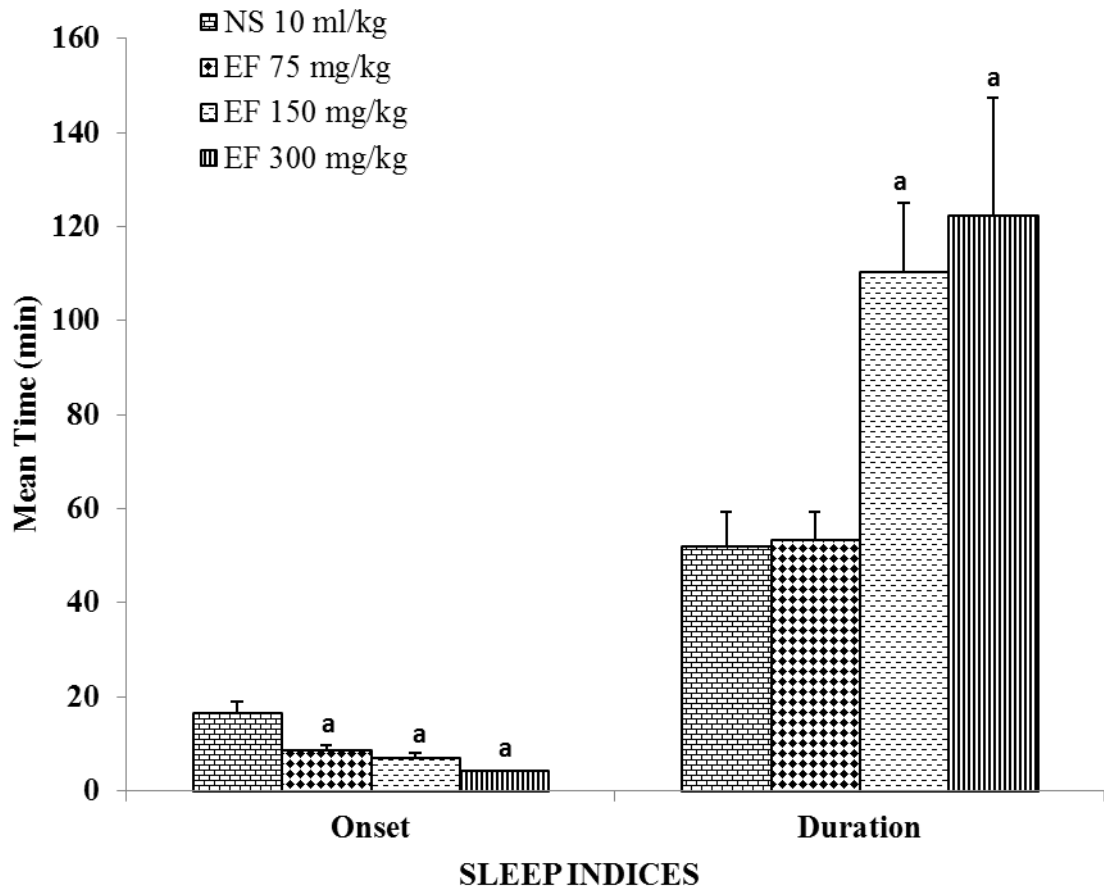
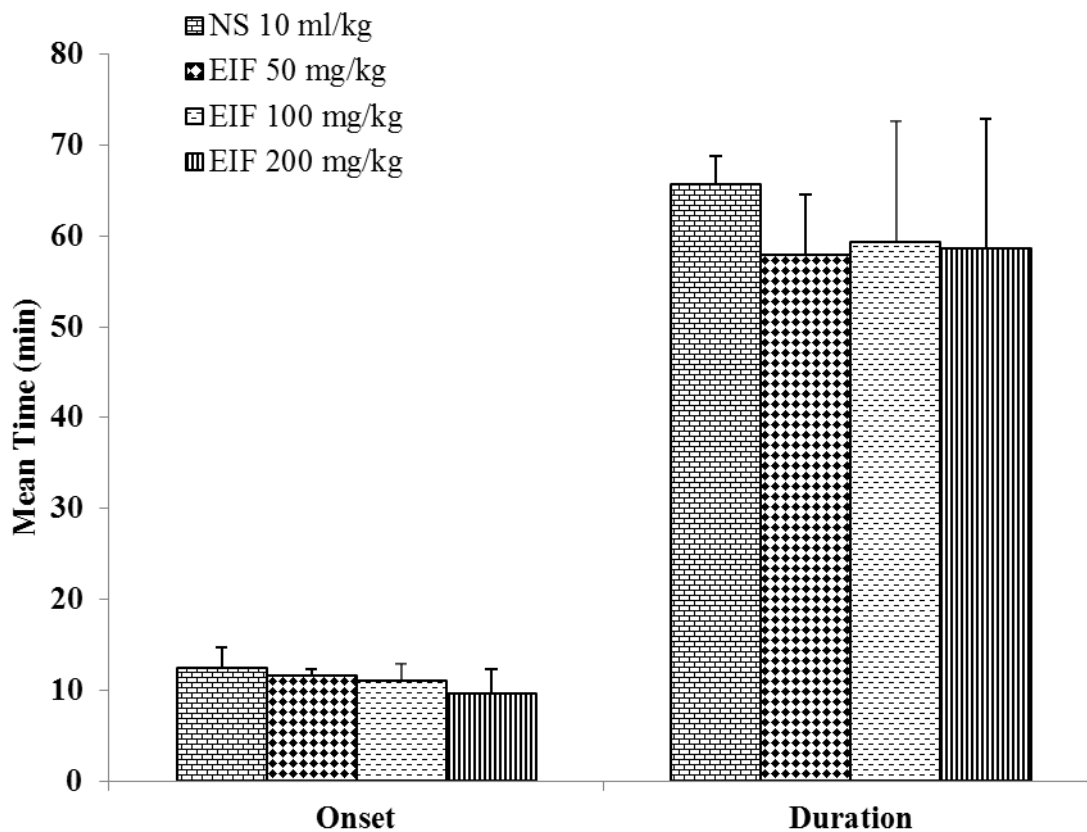
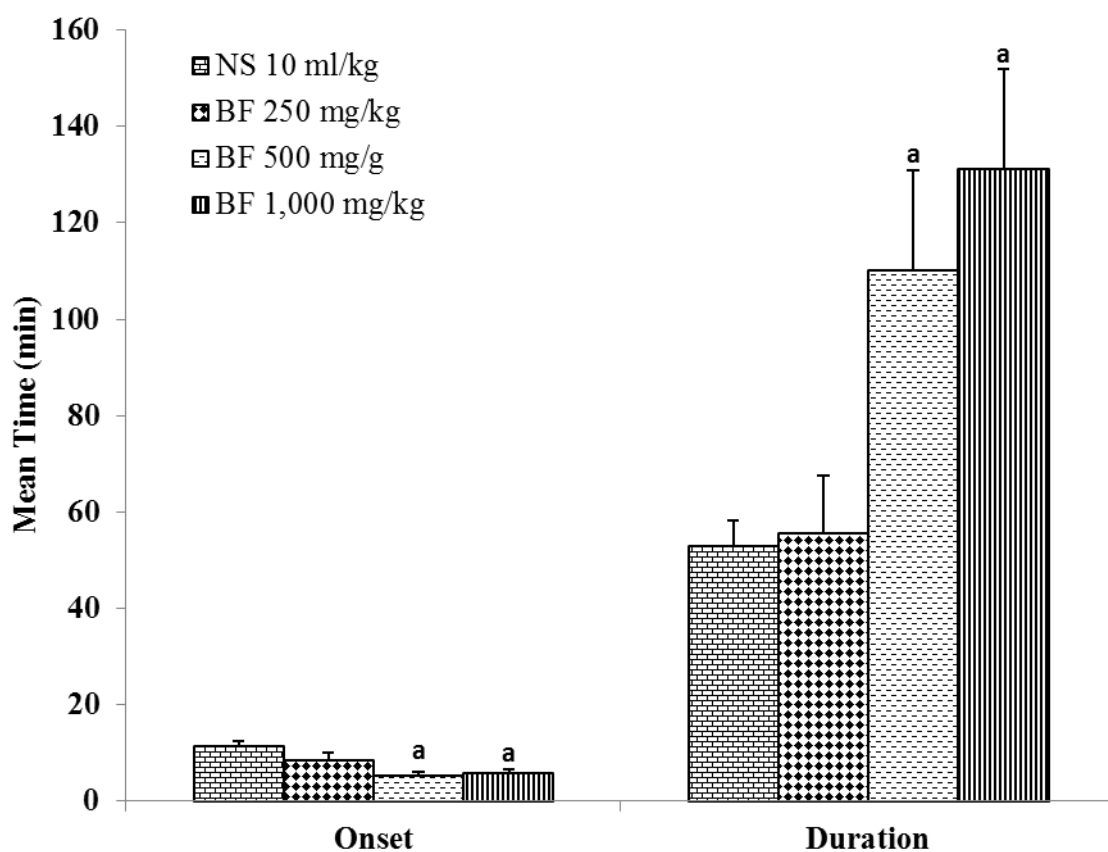


Figure 4.2: Effect of Ethylacetate Fraction on Onset and Duration in Diazepam-induced Sleep in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, Diazepam (25 mg/kg), route of administration = intraperitoneal, EF = Ethylacetate Fraction, NS = Normal saline.



SLEEP INDICES

Figure 4.3: Effect of Ethylacetate Insoluble Fraction on Onset and Duration in Diazepam-induced Sleep in Mice. No significant difference between control and treated groups, one way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean \pm SEM, Diazepam (25 mg/kg), route of administration = intraperitoneal, EIF = Ethylacetate Insoluble Fraction, NS = Normal saline.



SLEEP INDICES

Figure 4.4: Effect of Butanol Fraction on Onset and Duration in Diazepam-induced sleep in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, Diazepam (25 mg/kg), route of administration = intraperitoneal, BF = Butanol Fraction, NS = Normal saline.

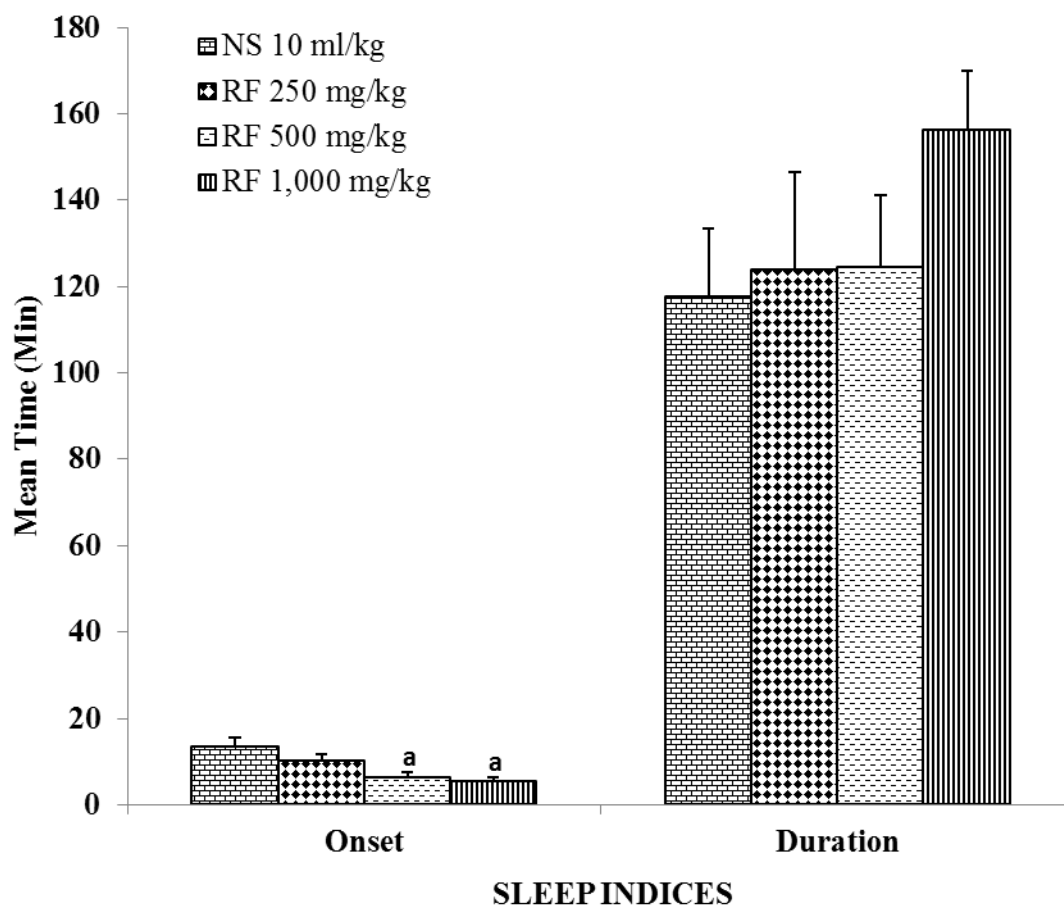


Figure 4.5: Effect of Residual Fraction on Onset and Duration in Diazepam-induced Sleep in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, Diazepam (25 mg/kg), route of administration = intraperitoneal, RF = Residual Fraction, NS = Normal saline.

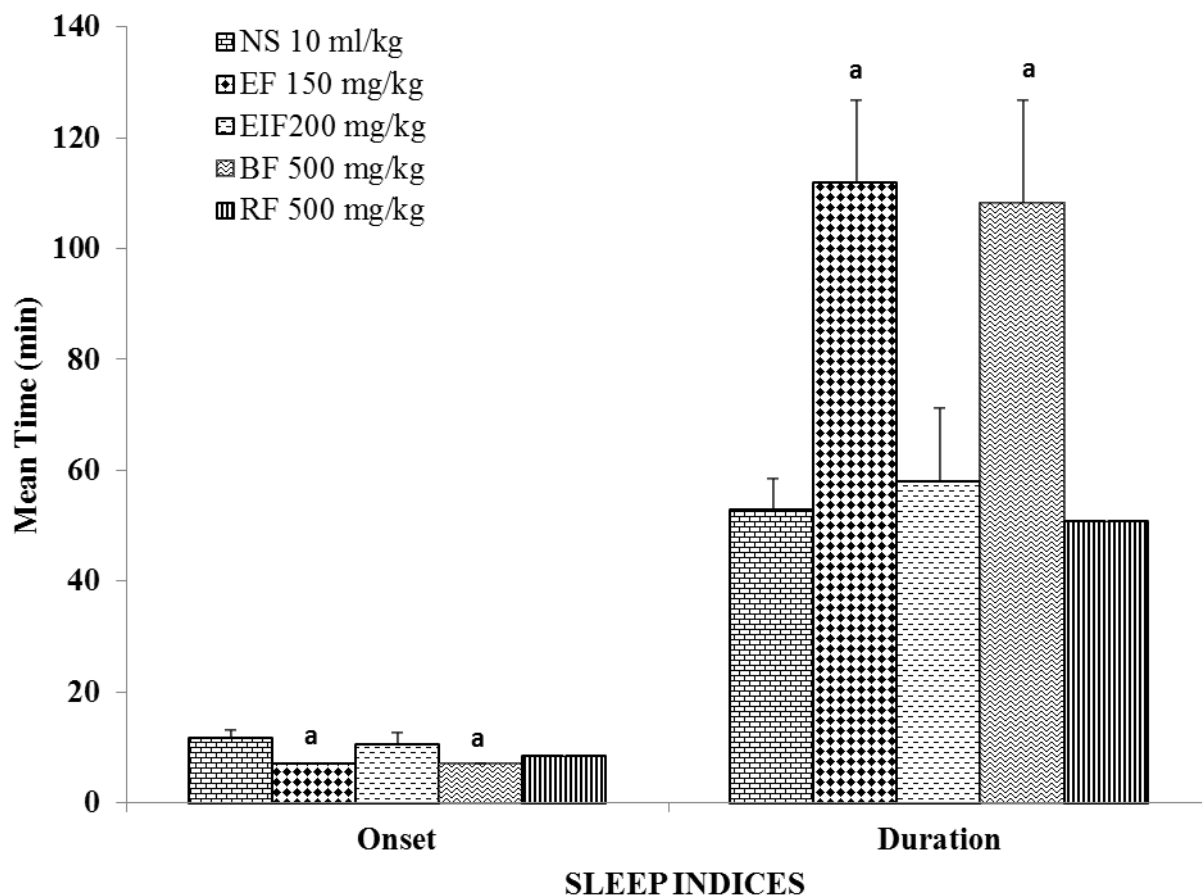


Figure 4.6: Comparative Effect of Fractions on Onset and Duration in Diazepam-induced Sleep in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, Diazepam (25 mg/kg), EF = Ethylacetate Fraction, EIF = Ethylacetate Insoluble Fraction, BF = Butanol Fraction, RF = Residual Fraction, NS = Normal saline.

4.4.2 Effect of ethanol extract and fractions of *T. globiferus* on exploratory behaviour in mice using hole-board test

The ethanol extract of *T. globiferus* at tested doses (87.5, 175 and 350 mg/kg) and diazepam (2 mg/kg) produced a significant ($p \leq 0.05$) decrease in the number of head dips in the hole-board experiment compared to control (Fig. 4.7).

Ethylacetate fraction and diazepam showed a significant ($p \leq 0.05$) decrease in the number of head dips at doses of 300 and 1 mg/kg respectively in the hole-board test compared to control treated with 10 ml/kg normal saline (Fig. 4.8).

Butanol fraction produced insignificant ($p \geq 0.05$) dose dependent decrease in the number of head dips in mice at tested doses (250, 500 and 1,000 mg/kg) while diazepam (2 mg/kg) showed significant ($p \leq 0.05$) decrease in the number of head dips in hole-board experiment compared to control (Fig. 4.9).

Exploratory activities of the diazepam, EF and BF were compared in which EF produced significant ($p \leq 0.05$) reduction in the number of head dips in mice followed by diazepam and then BP (Fig. 4.10).

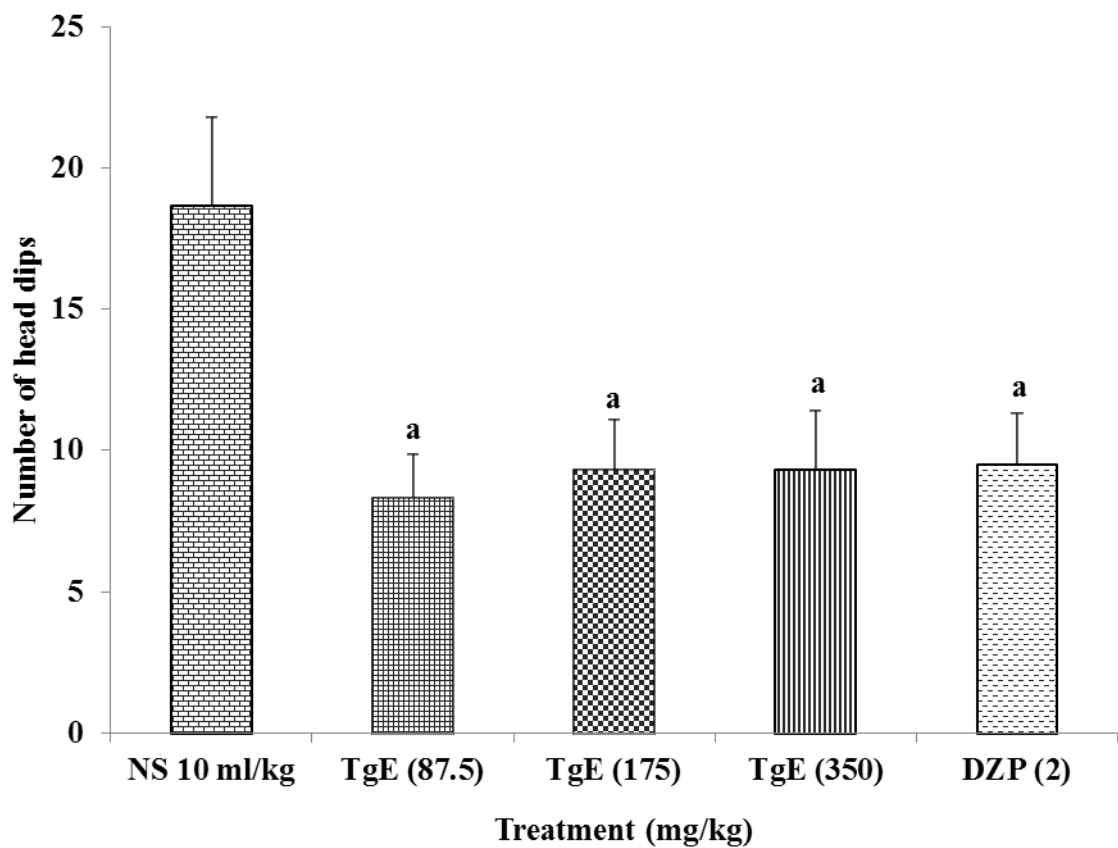


Figure 4.7: Effect of Ethanol Extract of *T. globiferus* on Exploratory Activity in Hole-board Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

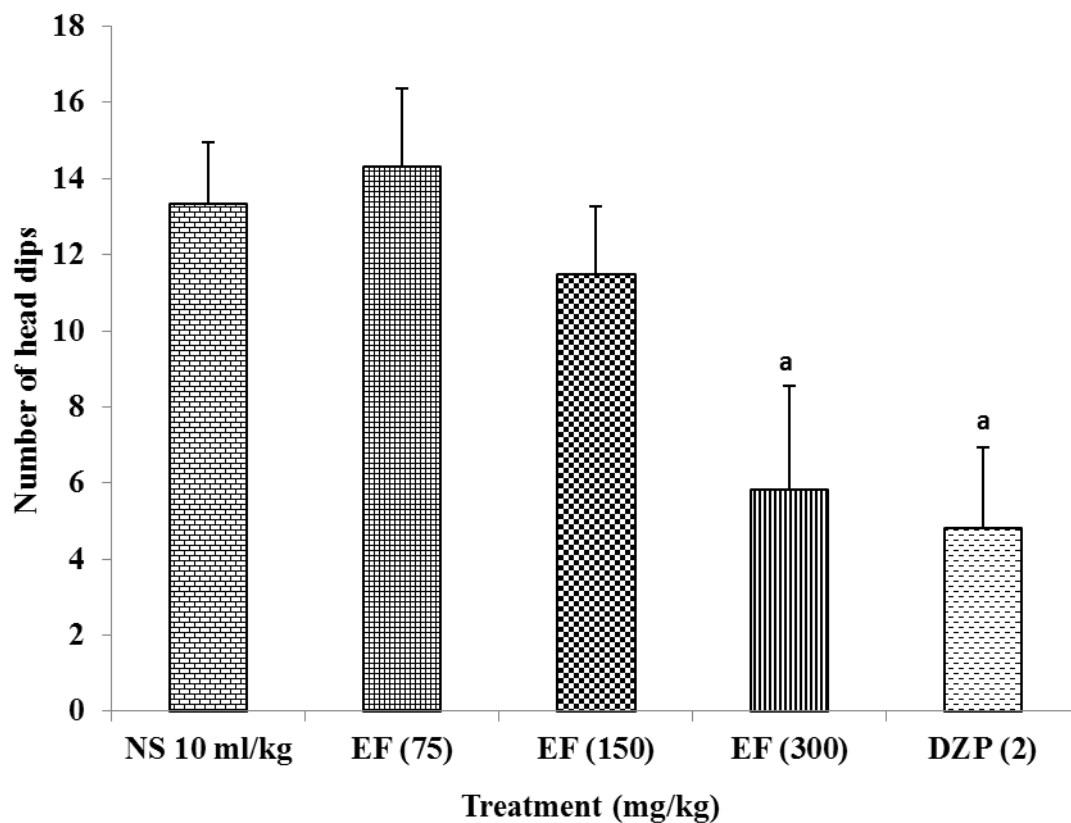


Figure 4.8: Effect of Ethylacetate Fraction of *T. globiferus* on Exploratory Activity in Hole-board Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, EF = Ethylacetate Fraction, NS = Normal saline, DZP = Diazepam.

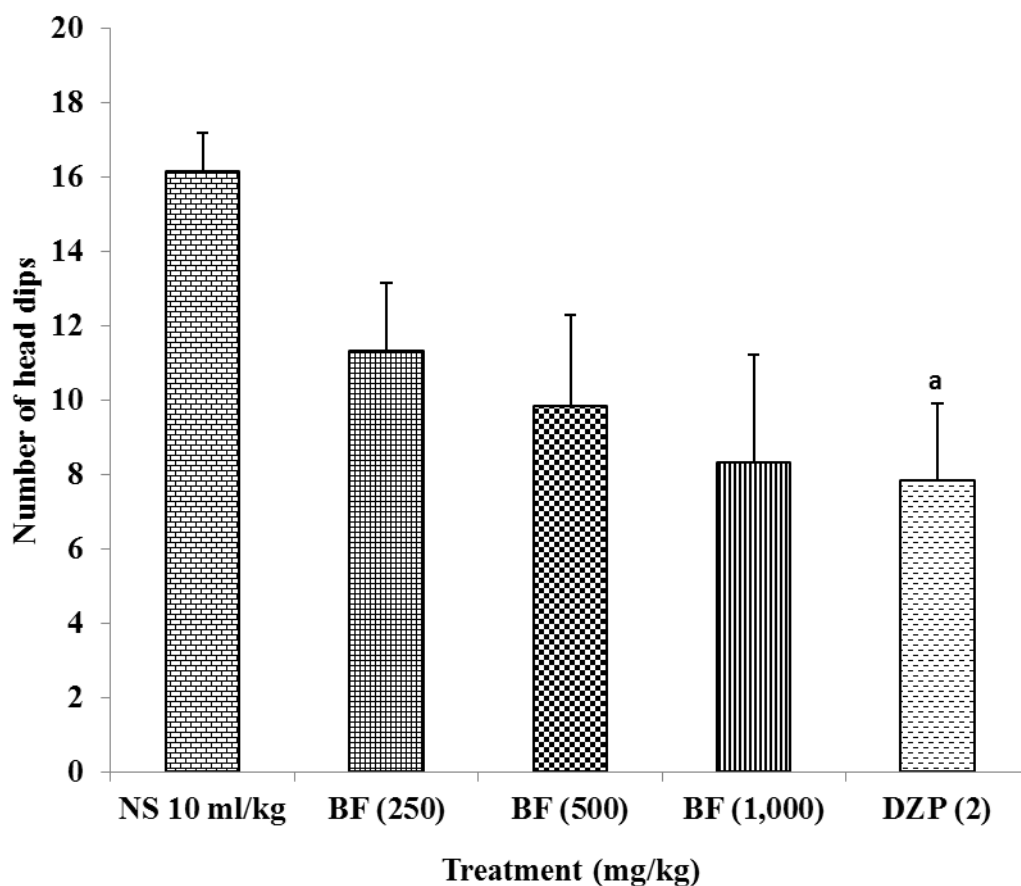


Figure 4.9: Effect of Butanol Fraction of *T. globiferus* on Exploratory Activity in Hole-board Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, BF = Butanol Fraction, NS = Normal saline, DZP = Diazepam.

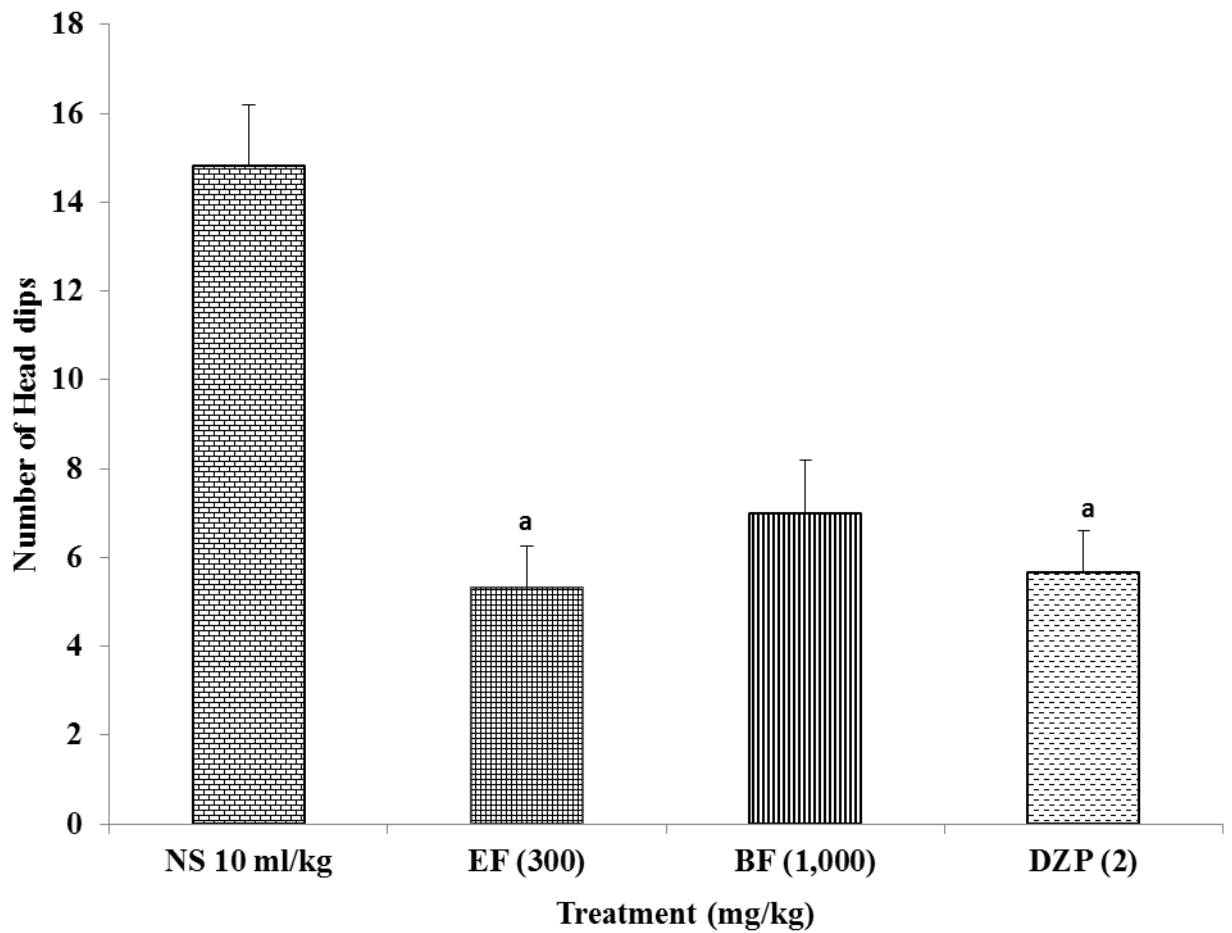


Figure 4.10: Comparative Effect of *T. globiferus* Fractions and Diazepam on Exploratory Activity in Hole-board Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. EF = Ethylacetate Fraction, BF = Butanol Fraction, $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, NS = Normal saline, DZP = Diazepam.

4.4.3 Effect of ethanol extract and fractions of *T. globiferus* on motor coordination using beam walking assay in mice

The ethanol extract of *T. globiferus* at the dose of 350 mg/kg offered significant ($p \leq 0.05$) increase in the time spent to reach the goal box and number of foot slips in mice compared to control. Diazepam (1 mg/kg) significantly ($p \leq 0.05$) increase the time spent to reach the goal box and number of foot slips in mice compared to the control (Fig. 4.11).

Ethylacetate fraction (EF) exhibited insignificant ($p \geq 0.05$) increase in the time spent on beam at tested doses of 75, 150 and 300 mg/kg and significantly ($p \leq 0.05$) increased the number of foot slips in mice at a dose of 300 mg/kg compared to control. However, diazepam (1 mg/kg) produced significant ($p \leq 0.05$) increase in the time spent to reach the goal box and number of foot slips compared to the control group (Fig. 4.12).

Butanol fraction (BF) produced an insignificant ($p \geq 0.05$) increase in the time spent to complete the task and number of foot slips in mice at tested doses (250, 500 and 1,000 mg/kg) compared to control. Diazepam (1 mg/kg) offered a significant ($p \leq 0.05$) increase in the number of foot slips compared to control (Fig. 4.13).

Effect of diazepam, EF and BF on motor coordination was compared in mice and showed that, diazepam produced significant ($p \leq 0.05$) increase in the time spent on beam and number of foot slips followed by EF and then BF (Fig. 4.14)

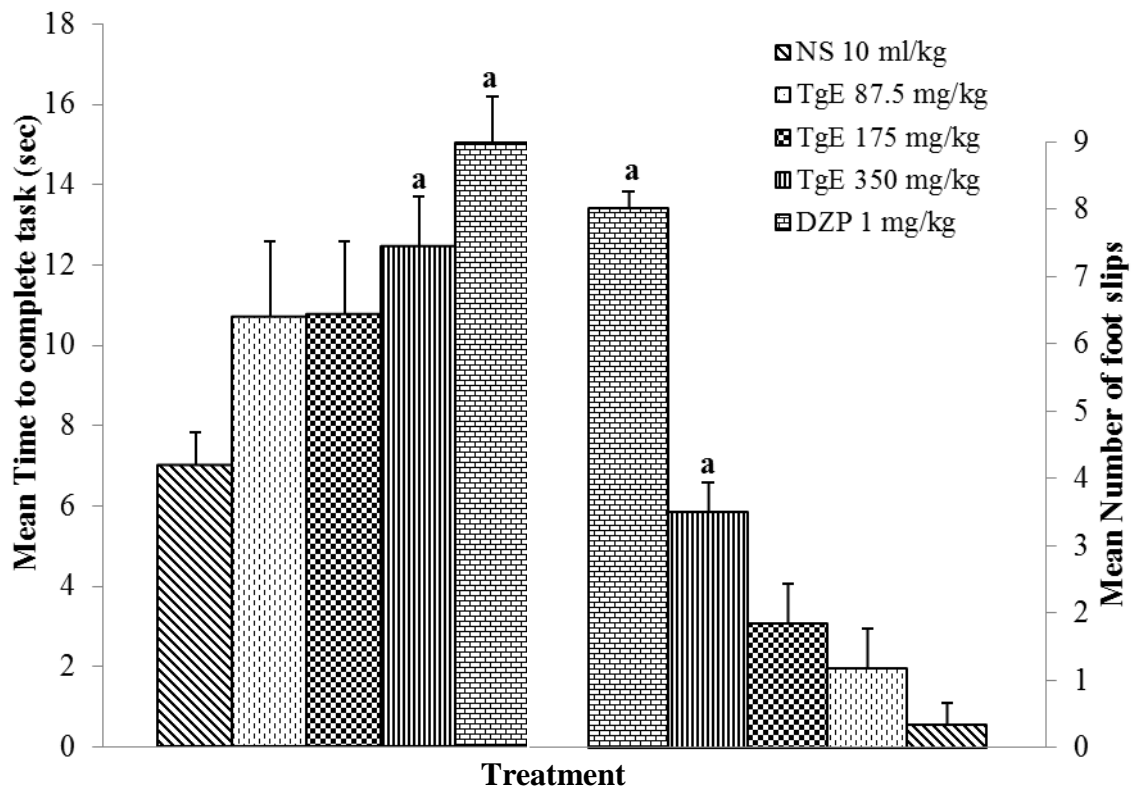


Figure 4.11: Effect of Ethanol Extract of *T. globiferus* on Time Spent and Number of Foot Slips in Beam Walk Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

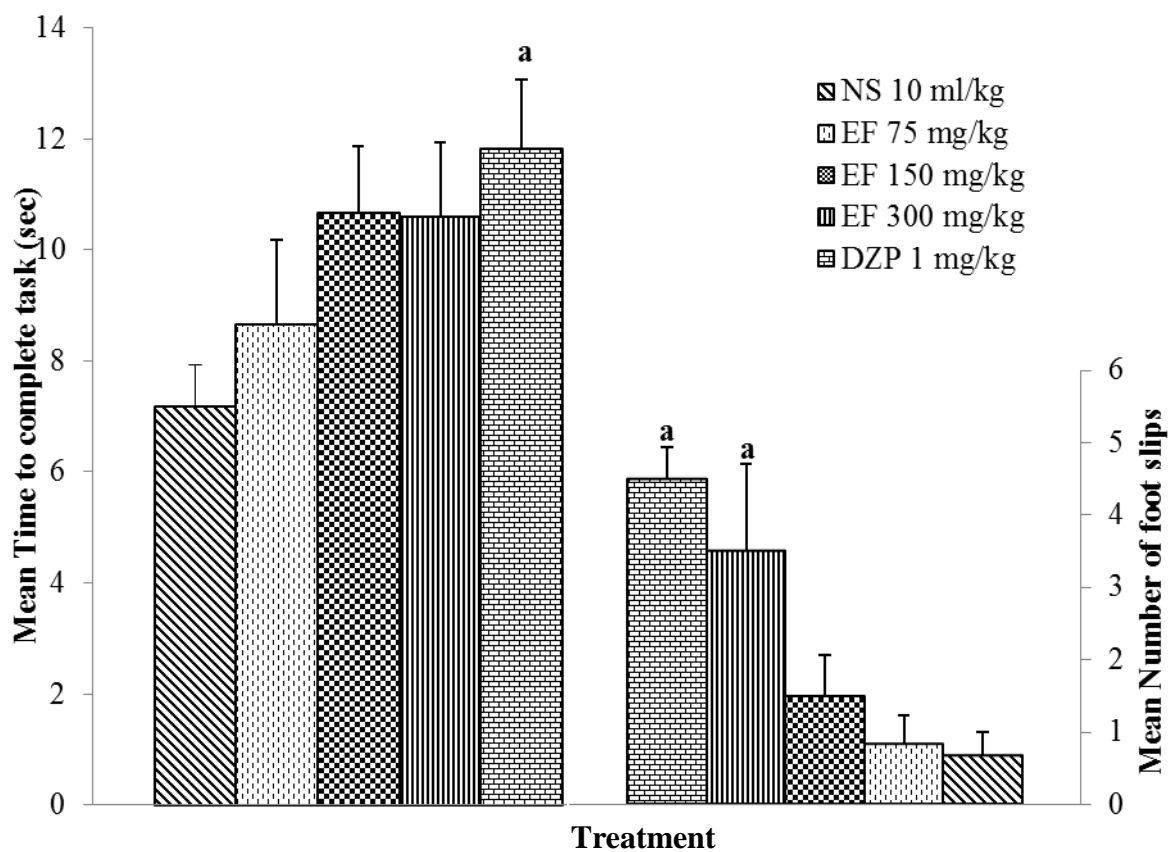


Figure 4.12: Effect of Ethylacetate Fraction of *T. globiferus* on Time Spent and Number of Foot Slips in Beam Walk Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, EF = Ethylacetate Fraction, NS = Normal saline, DZP = Diazepam.

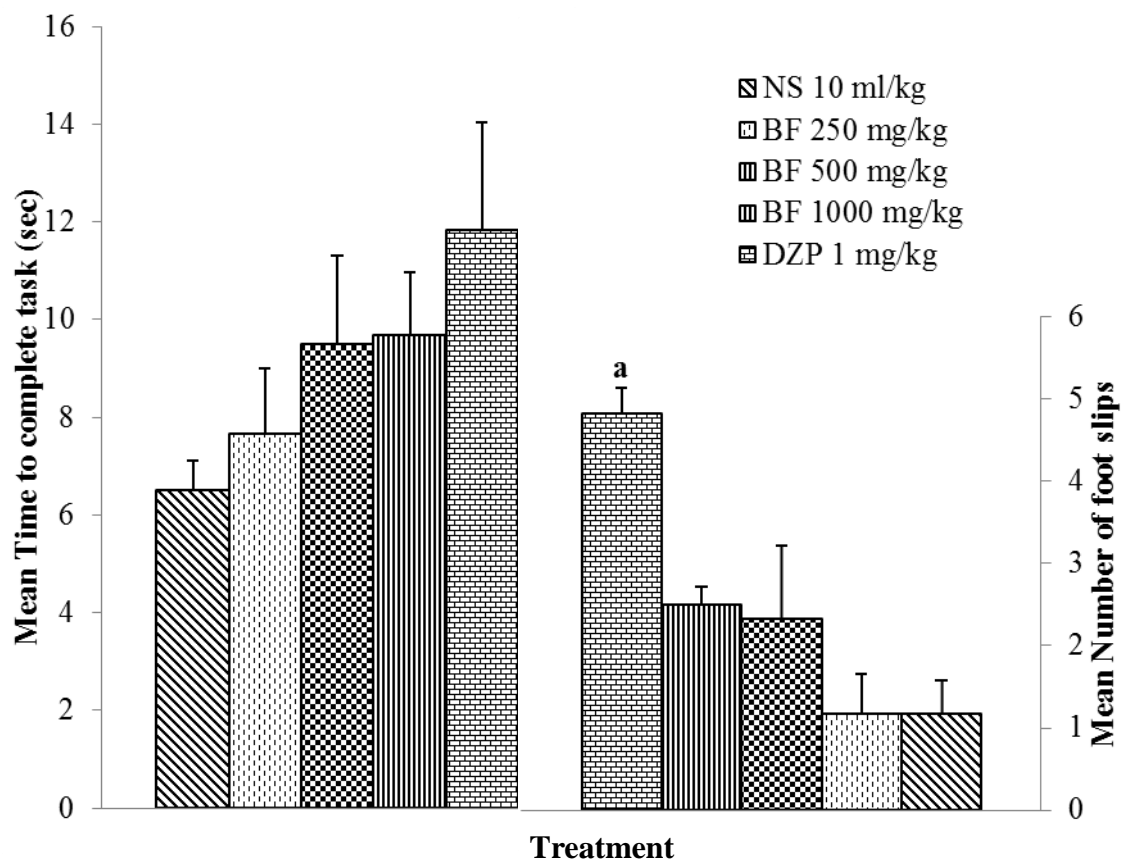


Figure 4.13: Effect of Butanol Fraction of *T. globiferus* on Time Spent and Number of Foot Slips in Beam Walk Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, BF = Butanol Fraction, NS = Normal saline, DZP = Diazepam.

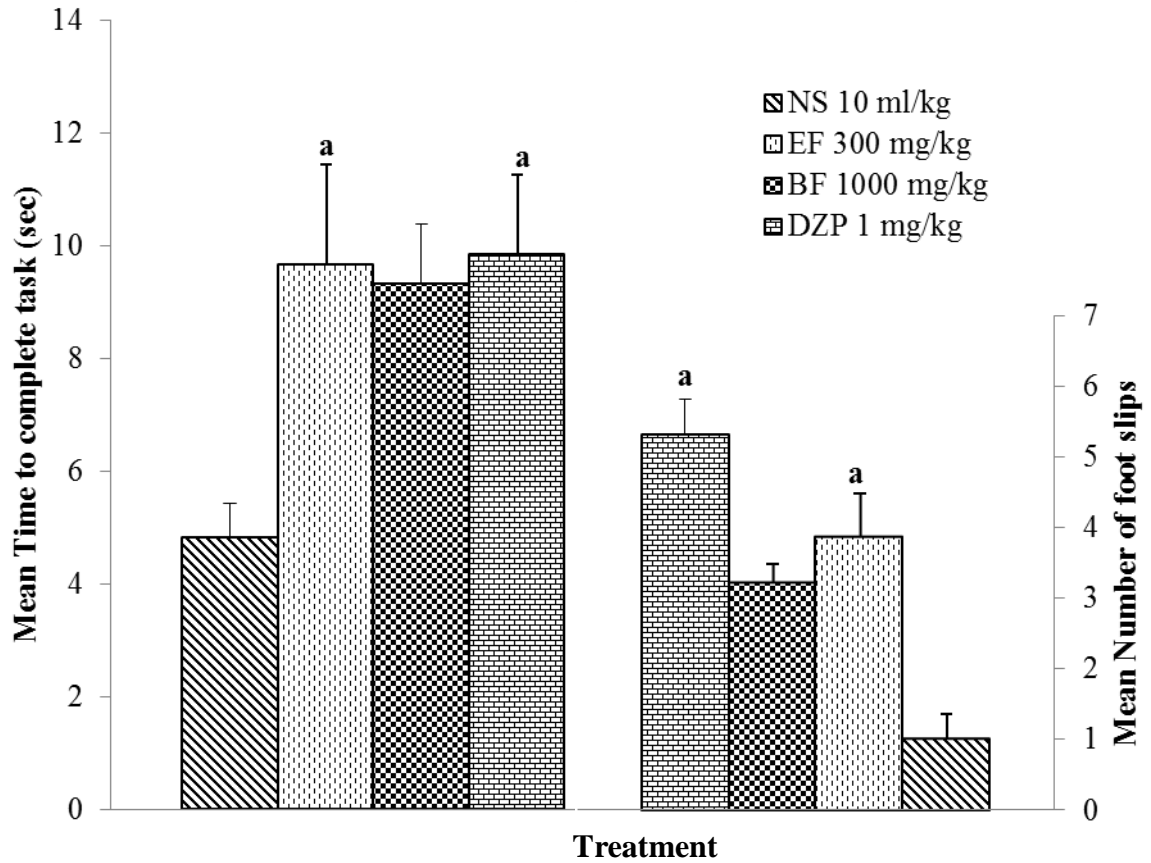


Figure 4.14: Comparative Effect of *T. globiferus* Fractions and Diazepam on Time Spent and Number of Foot Slips in Beam Walk Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, EF = Ethylacetate Fraction, BF = Butanol Fraction, NS = Normal saline, DZP = Diazepam.

4.4.4 Effect of ethanol extract of *T. globiferus* on elevated plus-maze test in mice

The ethanol extract of *T. globiferus* significantly ($p \leq 0.05$) increased the number of entries into the open arm only at a dose of 87.5 mg/kg when compared with control group treated with 10 ml/kg normal saline. However, diazepam (0.25 mg/kg) significantly ($p \leq 0.05$) increased both the time spent in the open arm and the number of entries into the open arm of elevated plus-maze test in mice compared to the control (Table 4.8).

4.4.5 Effect of ethanol extract of *T. globiferus* on staircase test in mice

The ethanol extract of *T. globiferus* produced a significant ($p \leq 0.05$) decrease in the number of steps climbed by mice in staircase test at dose of 350 mg/kg and produced an insignificant ($p \geq 0.05$) decrease in the number of rearing at tested doses (87.5, 175 and 350 mg/kg) compared to control treated with 10 ml/kg normal saline. However, diazepam used as positive control significantly ($p \leq 0.05$) decreased the number of steps climbed as well as number of rearing when compared to control group (Fig. 4.15).

4.4.6 Effect of ethanol extract of *T. globiferus* on open field test in mice

The ethanol extract of *T. globiferus* caused significant ($p \leq 0.05$) decreased in the number of lines crossed and number of rearing in the open field test at doses of 175 and 350 mg/kg respectively compared to control treated with 10 ml/kg normal saline. However, diazepam (standard drug) at dose of 1 mg/kg significantly ($p \leq 0.05$) reduced the number of line crossed, rearing, central square entries and central square duration compared to control (Table 4.9).

Table 4.8: Effect of Ethanol Extract of *Tapinanthus globiferus* on Number of Entries and Time Spent in Open and Closed Arms of Elevated Plus-maze Test in Mice

Treatment (mg/kg)	Number of Entries		Time spent (sec)	
	Open	Closed	Open	Closed
NS 10 ml/kg	6.00 ± 0.52	13.50 ± 1.38	75.83 ± 7.43	164.17 ± 16.31
TgE (87.5)	11.50 ± 0.76 ^a	8.33 ± 1.12	82.50 ± 9.21	141.83 ± 9.64
TgE (175)	8.83 ± 1.54	7.83 ± 0.87 ^a	103.50 ± 10.67	106.00 ± 15.22 ^a
TgE (350)	9.00 ± 1.44	8.00 ± 1.55	106.00 ± 4.82	103.67 ± 13.17 ^a
DZP (0.25)	12.00 ± 1.03 ^a	8.33 ± 2.20	129.17 ± 16.00 ^a	70.17 ± 7.55 ^a

^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

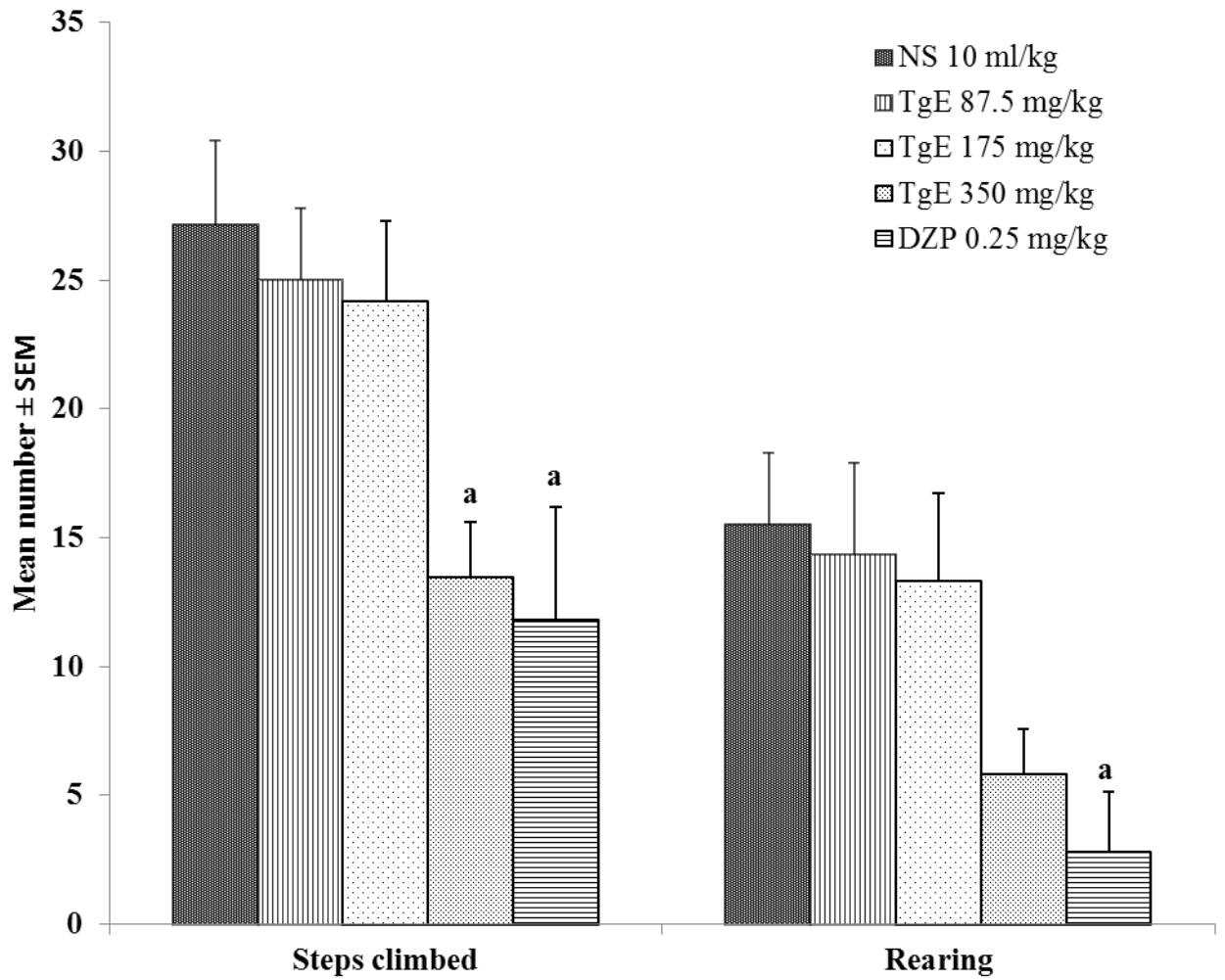


Figure 4.15: Effect of Ethanol Extract of *T. globiferus* on Number of Steps Climbed and Number of Rearing in Staircase Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

Table 4.9: Effect of Ethanol Extract of *Tapinanthus globiferus* on Behaviour of Mice in Open Field Test in Mice

Treatment (mg/kg)	LC	Rr	CSE	CSD (sec.)
NS 10 ml/kg	82.00 ± 19.15	23.83 ± 7.57	2.50 ± 0.76	0.08 ± 0.03
TgE (87.5)	77.17 ± 18.74	22.50 ± 8.04	3.33 ± 1.23	0.05 ± 0.01
TgE (175)	47.83 ± 4.14 ^a	8.67 ± 3.76	1.17 ± 0.31	0.08 ± 0.05
TgE (350)	53.17 ± 18.54	6.50 ± 0.83 ^a	1.67 ± 0.76	0.09 ± 0.25
DZP (1)	59.83 ± 2.38 ^a	7.00 ± 0.10 ^a	1.33 ± 0.07 ^a	0.01 ± 0.01 ^a

^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, LC = Line crossed, Rr = Rearing, CSE = Central square entries, CSD = Central square duration, DZP = Diazepam.

4.4.7 Effect of ethanol extract of *T. globiferus* on haloperidol-induced catalepsy in mice

The ethanol extract of *T. globiferus* offered significant ($p \leq 0.05$) potentiation in the duration of haloperidol-induced catalepsy in mice at dose of 350 mg/kg compared to control received 10 ml/kg normal saline (Table 4.10).

4.4.8 Effect of ethanol extract of *T. globiferus* on apomorphine-induced climbing behaviour in mice

The ethanol extract of *T. globiferus* at doses of 87.5 and 175 mg/kg produced significant ($p \leq 0.05$) reduction in the climbing score in mice induced by apomorphine (10 minutes after administration) compared to control (Table 4. 11).

4.4.9 Effect of ethanol extract of *T. globiferus* on tail suspension test in mice

The ethanol extract of *T. globiferus* at doses of 87.5, 175 and 350 mg/kg did not exhibit any significant ($p \geq 0.05$) effect on duration of immobility in mice suspended by tail. Imipramine (4 mg/kg) used as positive control significantly ($p \leq 0.05$) reduced the duration of immobility in mice compared to control (Fig. 4.16).

Table 4.10: Effect of Ethanol Extract of *Tapinanthus globiferus* on Haloperidol-induced Catalepsy in Mice

Treatment (mg/kg)	Time spent on the horizontal bar (sec)			
	30	60	90	120
NS 10 ml/kg	84.83 ± 26.15	115.17 ± 25.82	106.83 ± 33.01	139.33 ± 26.80
TgE (87.5)	81.33 ± 32.00	113.83 ± 27.06	129.67 ± 28.85	114.67 ± 34.70
TgE (175)	94.83 ± 38.12	145.17 ± 24.61	143.17 ± 28.33	133.17 ± 21.76
TgE (350)	137.17 ± 21.21 ^a	143.50 ± 26.26	167.17 ± 12.83 ^a	180.00 ± 0.00 ^a

^a $p \leq 0.05$ compared to NS and no significant difference over time–repeated measure ANOVA followed by Bonferroni *post-hoc* test. n = 6, Data = Mean ± SEM, Haloperidol (1.0 mg/kg), route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline.

Table 4.11: Effect of Ethanol Extract of *Tapinanthus globiferus* on Apomorphine-induced Climbing Behaviour in Mice

Treatment (mg/kg)	Climbing behavioural scores		
	10	20	30
NS 10 ml/kg	2.00 ± 0.00	2.00 ± 0.00	2.00 ± 0.00
TgE (87.5)	0.83 ± 0.40	1.33 ± 0.42	1.00 ± 0.37
TgE (175)	0.17 ± 0.17 ^a	1.33 ± 0.42	0.33 ± 0.33 ^a
TgE (350)	1.00 ± 0.45	1.33 ± 0.42	1.17 ± 0.40

^a $p \leq 0.05$ compared to NS, non-parametric test; Kruskal-Wallis followed by Duni's test. n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, Apomorphine (3 mg/kg s.c).

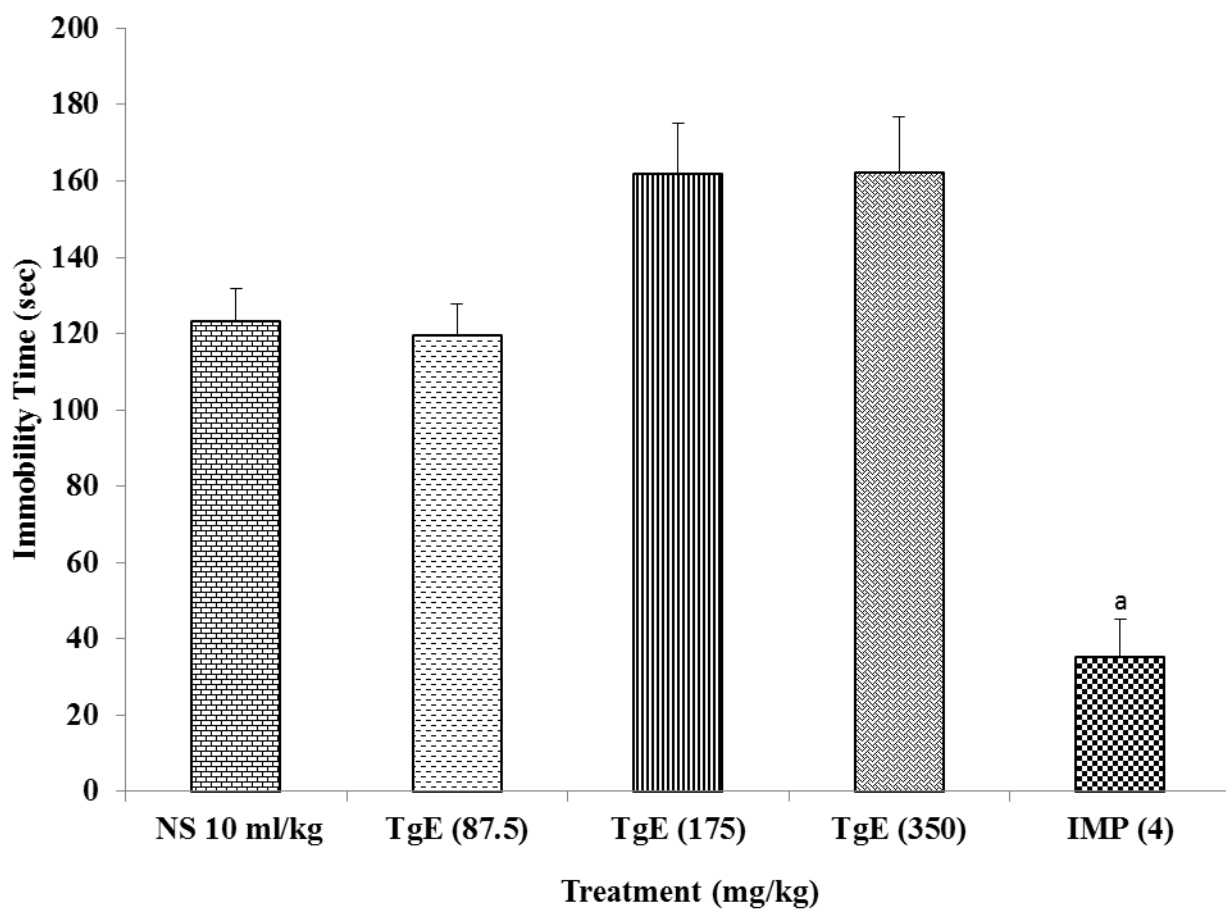


Figure 4.16: Effect of Ethanol Extract of *T. globiferus* on Immobility Time in Tail Suspension Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, IMP = Imipramine.

4.4.10 Effect of ethanol extract of *T. globiferus* on pentylenetetrazole-induced convulsion test in mice

The ethanol extract of *T. globiferus* at doses of 87.5, 175 and 350 mg/kg showed 16.67 and 33.33 % protection against subcutaneous pentylenetetrazole-induced seizure in mice. An insignificant ($p \geq 0.05$) delay in the onset of seizure was observed at the tested doses compared to the control group treated with 10 ml/kg normal saline. Sodium valproate (200 mg/kg) produced 100 % protection against pentylenetetrazole-induced convulsion (Table 4.12).

4.4.11 Effect of ethanol extract of *T. globiferus* on strychnine-induced convulsion test in mice

The ethanol extract of *T. globiferus* did not protect mice against strychnine induced convulsion at all tested doses (87.5, 175 and 350 mg/kg) but insignificantly ($p \geq 0.05$) prolonged the onset of convulsion at dose of 350 mg/kg when compared to the control group. Phenobarbitone (30 mg/kg) used as positive control produced 100 % protection against both convulsion and mortality induced by subcutaneous administration of 1.2 mg/kg strychnine (Table 4.13).

4.4.12 Effect of ethanol extract of *T. globiferus* on maximal electroshock-induced convulsion in chicks

The ethanol extract of *T. globiferus* offered no protection against hind limb tonic extension (HLTE) in maximal electroshock-induced convulsion in chicks at all tested doses (87.5, 175 and 350 mg/kg). Similarly, there was no significant ($p \geq 0.05$) difference in the recovery time at all tested doses of the extract used compared to the control. Phenytoin (20 mg/kg) produced 90 % protection against HLTE and showed

significant ($p \leq 0.05$) effect in mean recovery time of convulsed chicks compared to control (Table 4.14).

4.4.13 Effect of ethanol extract of *T. globiferus* on picrotoxin-induced convulsion test in mice

The ethanol extract of *T. globiferus* (87.5, 175 and 350 mg/kg) dose dependently protected 16.67, 33.33 and 50 % of the mice against picrotoxin-induced convulsion respectively and insignificantly ($p \geq 0.05$) decreased the onset of seizure compared to the control. Phenobarbitone (30 mg/kg) also produced 100 % protection against picrotoxin-induced convulsion in mice (Table 4.15).

4.4.14 Effect of bicuculline on activities of diazepam and ethylacetate fraction of *T. globiferus* on hole-board test in mice

Bicuculline (5 mg/kg) produced significant ($p \leq 0.05$) increase in the number of head dips in hole-board test in mice while diazepam (2 mg/kg) and ethylacetate fraction (300 mg/kg) significantly ($p \leq 0.05$) decreased number of head dips compared to group treated with 10 ml/kg normal saline (Fig. 4.17).

4.4.15 Effect of ethylacetate fraction of *T. globiferus* on ketamine-induced sleep in mice

Ethylacetate fraction of *T. globiferus* did not produce significant effects in the mean onset and the mean duration of sleep induced by ketamine at the doses tested (75, 150 and 300 mg/kg) compared to control received 10 ml/kg of normal saline (Fig. 4.18).

Table 4.12: Effect of Ethanol Extract of *Tapinanthus globiferus* on Pentylene-tetrazole-induced Convulsion in Mice

Treatment (mg/kg)	Mean onset of seizure (min)	Quantal protection	% protection	% mortality
NS 10 ml/kg	2.33 ± 0.56	1/6	16.67	83.33
TgE (87.5)	2.67 ± 0.61	1/6	16.67	83.33
TgE (175)	3.50 ± 1.15	2/6	33.33	66.67
TgE (350)	3.33 ± 1.09	2/6	33.33	50.00
SV (200)	0.00 ± 0.00 ^a	6/6	100.00	0.00

^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, SV = Sodium Valproate, Pentylene-tetrazole (75 mg/kg s.c).

Table 4.13: Effect of Ethanol Extract of *Tapinanthus globiferus* on Strychnine-induced Convulsion in Mice

Treatment (mg/kg)	Mean onset of convulsion (min)	Quantal protection	% protection	% mortality
NS 10 ml/kg	3.67 ± 0.21	0/6	0.00	100
TgE (87.5)	3.17 ± 0.48	0/6	0.00	100
TgE (175)	3.50 ± 0.43	0/6	0.00	100
TgE (350)	4.17 ± 0.60	0/6	0.00	100
PBT (30)	0.00 ± 0.00 ^a	6/6	100	0

^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, PBT = Phenobarbitone, Strychnine (1.2 mg/kg s.c).

Table 4.14: Effect of Ethanol Extract of *Tapinanthus globiferus* on Maximal Electroshock-induced Convulsion in Chicks

Treatment (mg/kg)	Mean recovery time (min)	Quantal protection	% protection	% mortality
NS 10 ml/kg	5.40 ± 1.15	1/10	10	0
TgE (87.5)	7.20 ± 0.79	0/10	0	0
TgE (175)	5.90 ± 0.59	0/10	0	0
TgE (350)	6.00 ± 0.87	0/10	0	0
PNT (20)	0.00 ± 0.00 ^a	9/10	90	0

^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. n = 10, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, PNT = Phenytoin.

Table 4.15: Effect of Ethanol Extract of *Tapinanthus globiferus* on Picrotoxin-induced Convulsion in Mice

Treatment (mg/kg)	Mean onset of convulsion (min)	Quantal protection	% protection	% mortality
NS 10 ml/kg	13.83 ± 1.89	0/6	0.00	100.00
TgE (87.5)	14.33 ± 3.29	1/6	16.67	16.67
TgE (175)	10.83 ± 4.09	2/6	33.33	50.00
TgE (350)	7.86 ± 3.21	3/6	50.00	50.00
PBT (30)	0.00 ± 0.00 ^a	6/6	100.00	0.00

^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, PBT = Phenobarbitone, Picrotoxin (5 mg/kg s.c).

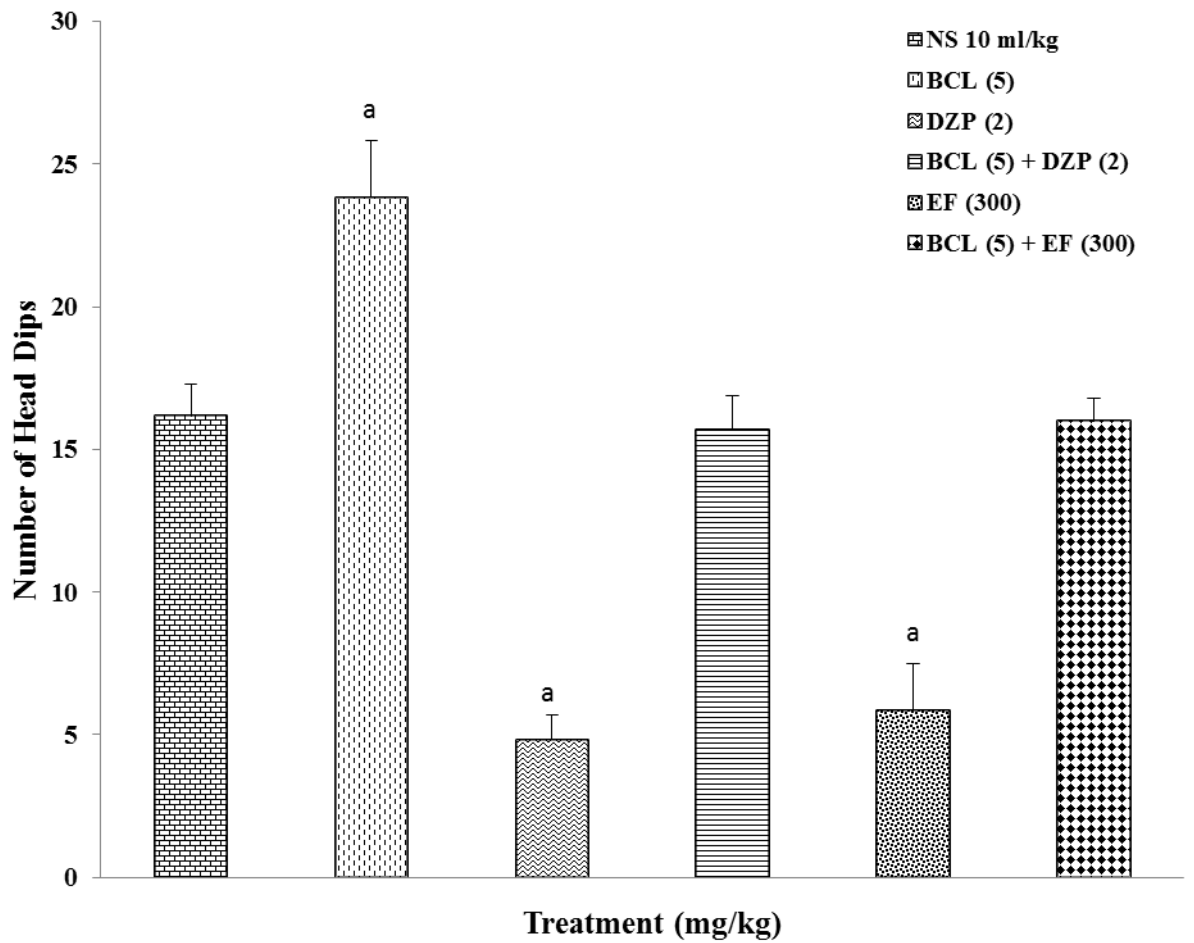


Figure 4.17: Effect of Bicuculline, Diazepam and Ethylacetate Fraction of *T. globiferus* on Hole-board Test in Mice. ^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, BCL = Bicuculline, DZP = Diazepam, EF = Ethylacetate Fraction NS = Normal saline.

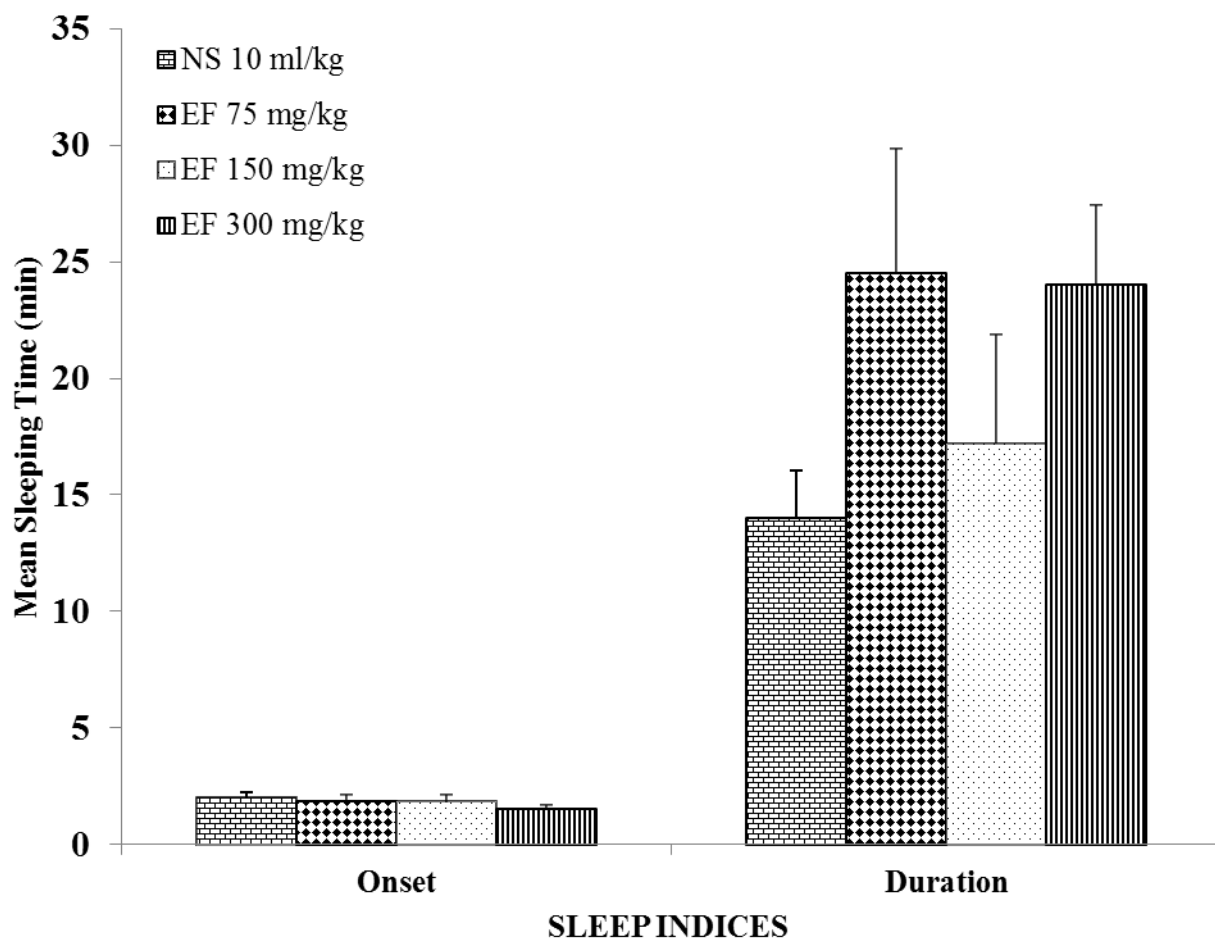


Figure 4.18: Effect of Ethylacetate Fraction of *T. globiferus* on Onset and Duration in Ketamine-induced Sleep in Mice. No significant difference between control and treated groups, one way ANOVA followed by Dunnett's *post-hoc* test. $n = 6$, Data = Mean \pm SEM, Ketamine (100 mg/kg), route of administration = intraperitoneal, EF = Ethylacetate Fraction, NS = Normal saline.

CHAPTER FIVE

5.0 DISCUSSION

Acute toxicity studies are designed to determine the dose that will produce high level of safety when given once and also serve to provide information regarding doses that would be used in sub-chronic or chronic studies (Arome and Chinedu, 2014). The values of LD₅₀ obtained showed that, the ethanol extract and fractions of *T. globiferus* are slightly toxic when administered intraperitoneally in mice and rats. This is according to Lorke (1983), the proposed scale of; LD₅₀ < 1.0 mg/kg-very toxic, LD₅₀ < 10 mg/kg-toxic, LD₅₀ up to 100 mg/kg- less toxic, LD₅₀ up to 5,000 mg/kg- slightly toxic and substance with LD₅₀ values greater than 5,000 mg/kg are practically non-toxic. However, oral administration of *T. globiferus* extract in mice and rats revealed that, the extract is practically non-toxic. This is based on the toxicity ratings by Matsumura (1975); Corbett *et al.* (1984) as the LD₅₀ value between 500 – 5,000 mg/kg slightly toxic and 5,000 – 15,000 mg/kg practically non-toxic.

The body weights observed from the rats were not affected by the ethanol extract of *T. globiferus* which probably suggest that the extract may not produce any toxic effect in the organs, following twenty eight (28) days daily oral treatment. Some studies showed that toxic substance produced significant effects in the body weight of rats by either increase or decrease as a result of organs or systems damage (Amna *et al.*, 2013; Ebadan *et al.*, 2014).

Haematological study is one of the important ways for the diagnosis of root cause of disease (Choudhari and Deshmukh, 2007). Haematological parameters such as packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), white blood cell (WBC),

neutrophil (Neut) and lymphocyte (Lymp) are used to provide useful information for diagnosis in routine clinical evaluation of the state of health of a patient (Berezi *et al.*, 2013). The observed reduction in PCV, Hb and RBC values indicate that, the extract may have been toxic to circulating cells and possibly interfered with RBC production which may lead to anaemia. Reduction in PCV and RBC values are indication of anaemia (Tilak *et al.*, 2007) and reduction in Lymphocyte values are indication of a non-enhanced immunological status (Guyton and Hall, 2006) hence, the extract lowered Lymphocyte values indicates that the immunological status of the body was not enhanced. The observed elevation of WBC values may be due to the stimulation of immune defence system by the extract. Stimulation of immune defence system may result in the elevation of WBC values (Berezi *et al.*, 2013). Some plant materials when ingested either in the raw state or their extract have been reported to cause anaemia which may result from sequestration of red blood cell in the spleen, impaired red cell production or primary bone marrow dysfunction (Choudhury and Sinha, 2015).

Increased in serum concentration of ALT and AST are indicative parameters of liver damage or disorder, but ALT is more specific to liver and thus a better parameter for detecting liver injury as AST is also associated with diseases of other organs such as heart and muscle (Ezeji *et al.*, 2014). This revealed that, the extract might have less effect on liver and other organs, because the increase in concentration of ALT and AST are not significant. The concentration of ALP was observed to remain unchanged at tested doses. ALP is present mostly in cells lining the biliary duct of the liver and is used to diagnose obstruction to the biliary system and its elevation in the blood indicates cholestatic diseases such as gallstone or tumour blocking the bile duct (Ezeji *et al.*, 2014). Serum total protein (TP) was observed to be unchanged at tested doses of

extract. Research has shown that an increase in serum TP is usually due to increase in globulin production, chronic infections or liver disease while decrease is associated with high tissue demands for protein, this is due to serious liver damage or liver disease associated with a reduction in protein synthesis, loss of protein from the body in urine and low protein intake (Kwete *et al.*, 2010). Thus, liver damage or disease may result in reduction of protein synthesis. The observed significant ($p \leq 0.05$) increase in serum total bilirubin (TB) concentration at tested doses might not be as a result of liver damage because there was an increase in the concentration of conjugated bilirubin (CB) at the same doses. The concentration of CB in the blood depends on TB, because the biotransformation of TB increases the concentration of blood CB. Bilirubin is a metabolic product formed from the breakdown of erythrocytes and increase in serum TB could be due to damage of the liver cells or hepatic diseases like jaundice and obstruction of the duct (Arthur and John, 1986). However, increased bilirubin concentrations reflect the depth of jaundice and an indication of brain cells damage (Ogunmefun *et al.*, 2013).

The serum concentration of urea, creatinine, sodium (Na^+) and chloride (Cl^-) were observed to change, may be as a result of the adaptation mechanism to physiological conditions. There was decrease in Na^+ , increase in Cl^- and K^+ concentration which may result in physiological action of the Na^+ - K^+ ATPase pump in trying to maintain the level of Na^+ and K^+ , and showed that ethanol extract of *T. globiferus* do not causing any damage to the kidney. Study showed that, the significant ($p \leq 0.05$) changes in rat biochemical levels may occur due to the maintenance of their concentration in the body (Ibrahim *et al.*, 2006). Electrolytes such as sodium, potassium, calcium, chloride and

biocarbonate are vital for the normal functioning of the body and they were excreted via kidney.

The degeneration of hepatocyte observed in the liver section of rats was correlated with result of biomarker test in which the serum concentration of ALT and AST was observed to increase at higher dose of extract. However, ALT is more specific in hepatotoxicity injury (Ezeji *et al.*, 2014). The toxicity of this extract at higher dose might be related to increase in the concentration of toxic chemical constituents. *T. globiferus* have been reported to contain alkaloids, flavonoids, saponins, tannins and cardiac glycosides (Bassey, 2012).

The degeneration of Bowman's capsule, tubular degeneration and blood vessel distortion observed in the kidney section of rats may be due to the acid-base imbalance of HCO_3^- and Cl^- , since biochemical analysis revealed the significant reduction in the serum concentrations of urea, creatinine and Na^+ . Increase in serum urea, creatinine and Na^+ is an indication of functional damage to the kidney (Garba *et al.*, 2007). However, urea, creatinine and electrolytes (Na^+ , K^+ , HCO_3^- and Cl^-) are the most sensitive biochemical markers employed in the diagnosis of renal function (Afolabi *et al.*, 2014) because urea and creatinine are excreted through the kidney while electrolytes are reabsorbed and excreted in the tubules. Therefore, tubular damage may lead to retention of urea and creatinine in the blood and non-reabsorption of electrolytes. However, the extract have no toxic effect on structural integrity and function of the rat kidney at doses of 87.5 and 175 mg/kg may be due the presence of less toxic chemical constituents, but showed tendency to predispose to kidney toxicity at the higher dose of 350 mg/kg.

The sign of toxic effect in the spleen of rats was coincided with haematological findings. However, the red pulp degeneration and necrosis of epithelial cell observed in spleen may lead to the significant change in red blood cell. Damage to the spleen may lead to accumulation of dead and abnormal cells, bacteria and other toxicants in the blood (Goerge-Gay and Parker, 2003). Spleen helps to destroy old red blood, form lymphocytes and serves as a reservoir of erythrocytes (Zandonai *et al.*, 2010; Choudhury and Sinha, 2015). The extract produced no effect in the serum levels of WBC, NEUT, LYMP, MONO and EOSI at all tested doses.

Generally, ethanol extract of *T. globiferus* was observed to show less or no sign of pathological effect in liver at dose of 87.5 mg/kg as well as kidney and spleen at doses of 87.5 and 175 mg/kg, but produced a sign of toxicity at higher dose of 350 mg/kg in the aforementioned organs as confirmed in the biochemical and haematological studies. Previous studies have also confirmed the non-toxicity of the *T. globiferus* extract at lower doses (Adekunle *et al.*, 2012; Abubakar *et al.*, 2016).

The ethanol extract and fractions of *T. globiferus* reduced the onset and prolonged the duration of sleep induced by diazepam in a dose dependent manner which an indication of central depression activity (Chindo *et al.*, 2014), the extract and fractions seems to possesses sleep inducing properties. The potentiation effects of the extract and fractions on diazepam-induced sleep, may suggests their central nervous system depressant activity which could be sedative. The sedative property may possibly related to the presence of some chemical components in the extract and fractions that activating the benzodiazepine or GABA receptors in the GABA_A receptor complex which may responsible for their potentiation activity or maintenance of sleep.

The comparative study carried out on the fractions revealed the higher significant ($p \leq 0.05$) effect of ethylacetate and butanol fractions on the onset and duration of sleep while ethylacetate insoluble and residual fractions produced insignificant ($p \geq 0.05$) effect on the onset and duration in diazepam-induced sleep. The activities observed in the fractions appeared to be more in the ethylacetate followed by butanol which may possibly due to the presence of alkaloids in ethylacetate fraction.

The extract and ethylacetate fraction (EF) produced decrease in exploratory behaviour by decrease in the number of head dip in hole-board test. Hole-board tests are used to measure exploratory behaviour and agent that decrease exploratory behaviour by reduction in head dips may have sedative activity while an increase in number of head dips reveals anxiolytic activity (Mandal *et al.*, 2001). Research showed that decrease in exploratory behaviour support neurosedative property (Adzu, 2002) and used to measure CNS depression (Tanko *et al.*, 2009). These may suggest that, the extract and EF might have CNS depressant activity. The activity of EF and butanol fraction (BF) was compared and found that, they significantly ($p \leq 0.05$) reduced the number of head dips. The reduction activity observed to be higher in EF then BF may be as a result of difference in alkaloids contents which was found to be absence in BF. The effect of fractions on the head dips may reveal the depression property similar to the activity observed in the diazepam (standard drug).

The increase in the number of foot slips in beam walk reveals that the extract may have motor coordination deficits activity. Beam-walking assay is used to test the effect of substance on motor coordination in laboratory animals (Stanley *et al.*, 2005) as well as to screen for peripheral neuromuscular blockade (Ya'u *et al.*, 2011) and is a sensitive

model for detecting sedative dose (Magaji *et al.*, 2012). The number of foot slips made in beam walking assay is a sensitive measure in detecting benzodiazepine-induced motor coordination deficits in mice and more useful in predicting doses that could cause sedation in clinical settings (Stanley *et al.*, 2005). The activity of EF and BF was also compared in beam walk and found that EF produces more effect in time spent and number foot slips on beam than BF, although time spent is less important measure because of falling and inability to reach the goal box, but increase in the number foot slips is a measure of sedative activity (Stanley *et al.*, 2005).

The effects of ethanol extract and fractions of *T. globiferus* on diazepam-induced sleep, hole-board test and beam walking assay suggest the CNS depressant property of the plant. Diazepam-induced sleep is a model used to assess sedative activity while beam-walking assay to distinguish central and peripheral activity (Ya'u *et al.*, 2011). The ability of the extract and fractions to potentiate diazepam-induced sleep, reduction in the exploratory behaviour in hole-board test and increased number of foot slips confirmed their depressant activity. They might have produced their depressant effects through activation of the receptors of endogenous neurotransmitters such as dopamine, serotonin, Gamma Amino Butyric Acid (GABA), histamine or neuropeptides. Alternatively, they potentiate the sedative property of diazepam and act by interacting with GABA-mediated synaptic transmission (Tanko *et al.*, 2009) which may probably acts directly on GABA_A receptors or enhancing the action of GABA on GABA_A receptors (Ritcher *et al.*, 2012). Sedative-hypnotic agents such as benzodiazepines and barbiturates act by enhancing the action of GABA on GABA receptor channel complex by binding on specific allosteric sites on GABA_A receptors to enhance GABA binding to the GABA receptor resulting to the potentiation of GABA responses (Johnston,

2005). The CNS depressant property may be due to the presence of alkaloids, flavonoids, terpenoids and saponins in the extract and fractions. Sedative effect of alkaloids, flavonoids and terpenoids obtained from *Byrsocarpus coccineus* extract was reported (Akindele and Adeyemi, 2010). Terpenoids and flavonoids have been reported to show potential sedative and anxiolytic activity in mice (Edewor-kuponiyi, 2013). Saponins are also reported to have potent sedative activity (Amos *et al.*, 2001; Adzu *et al.*, 2002).

The intraperitoneal administration of the ethanol extract of *T. globiferus* produced increase in the number of entries into open arms at lower dose. A significant ($p \leq 0.05$) increase in the number of entries and time spent in open arms of maze is a measure of anxiolytic property (Kavyasree *et al.*, 2013) and sedative at lower dose is anxiolytic which may suggest the sedative activity of the extract at low dose of 87.5 mg/kg. This is buttressed by the significant ($p \leq 0.05$) decrease in the number of steps climbed in staircase. This method is a measure of exploratory activity and reveals the sedative property of the ethanol extract of *T. globiferus*. The extract caused insignificant ($p \geq 0.05$) decrease in the number of rearing at tested doses. However, anxiolytic agents produce significant ($p \leq 0.05$) decrease in the number of rearing and no effect on number of steps climbed in mice. Reduction in mice rearing is a measure of anxiolytic property (Ya'u *et al.*, 2011). Similarly, diazepam (0.25 mg/kg) reduced the number of both steps climbed and rearing behaviour in mice. In staircase model, step climbing serves as an index of exploratory or locomotor activity while rearing serves as an index of anxiety (Vogel, 2008). However, the model is used to detect behavioural effects of agents active at the GABA_A receptors (Simiand *et al.*, 1984).

In the open field test in mice, the ethanol extract of *T. globiferus* produced significant reduction in the number of line crossed and rearing, and the reduction observed in these parameters may be due to CNS depression. However, measurement of several parameters in an open field allows to identifying various types of sedative or stimulant agents (Vogel, 2008). Study showed that reduction in the number of line crossed, grooming, rearing and central square entries predict the anxiolytic and hypnotic property (Hosseinzadeh *et al.*, 2012). Increase in number of line crossed (locomotion) can be considered a stimulant effect while decreased locomotion is related to sedation (Belzung and Prut, 2003); hence the extract decreased the number of line crossed. This could be as a possible sedative effect of the extract and it could have anxiolytic properties at lower doses.

Staircase and open field tests are useful in identifying specific and non-specific effects of drug and assessing anxiety as well as aspects of sedation (Ya'u *et al.*, 2011). Mice are preference to stay close to the walls in open field is a natural tendency of been anxious. The number of entries into the central and the time spent in the central square are measures of anxiolytic (Sethi *et al.*, 2005). The pathophysiology of anxiety disorders had been observed to be due to the neurobiological abnormalities of endogenous neurotransmitters in serotonergic, noradrenergic, glutamatergic and GABAergic transmission (Nutt and Malizia, 2001). Activation of GABA receptors directly or indirectly would have anxiolytic effect, given that GABA_A receptors are implicated in anxiety disorders (Vogel and Vogel, 2002). However, the anxiolytic activity of *T. globiferus* extract may be due to sedative activity on the receptors of the endogenous neurotransmitters. Probably, the extract may produce it effect through GABA_A receptor. Agents active at the GABA_A receptor complex have been shown to reduce rearing at

doses that do not reduce climbing in the staircase test (Simiand *et al.*, 1984). Natural and synthetic flavonoids are found to be potent anxiolytic agents without sedative effects (Herberlain *et al.*, 1994).

The ethanol extract of *T. globiferus* potentiated haloperidol-induced catalepsy in mice which revealed that, the extract have antipsychotic property. Haloperidol is a neuroleptic compound which is observed to act as a D₂ receptor antagonist in the mesolimbic-mesocortical and nigrostriatal pathways (Pathan *et al.*, 2009) and agents that increase dopamine transmission would inhibits neuroleptic-induced catalepsy (Yadav and Nade, 2008). An agent would induce catalepsy when it can antagonise more than 80% of D₂ receptors (Wadenberg *et al.*, 2001) in the nigrostriatal pathway.

The ethanol extract of *T. globiferus* initiate reduction in climbing behaviour induced by apomorphine which revealed the antagonist effect of the extract on apomorphine receptor. The climbing behaviour observed in mice after apomorphine administration is attributed to activation of D₁ and D₂ receptors (Stoff and Keabian, 1984). Apomorphines induce climbing behaviour by direct stimulation of postsynaptic striatal and mesolimbic dopamine receptors (Costall *et al.* 1978). Antipsychotic drugs antagonise dopamine D₂-receptors and are clinically more effective against hallucinations and delusions (Gardner *et al.*, 2005). Reduction in apomorphine-induced stereotypic climbing behaviour in rodents is one of the properties of antipsychotic that might act as dopamine D₁ and D₂ receptor blockade (Pandy *et al.*, 2012). The observed effect of the extract on haloperidol-induced catalepsy and significant reduction in climbing behaviour induced by apomorphine showed that the extract may have effect on dopamine receptors. Haloperidol is a dopamine receptor antagonist while apomorphine

is a short acting central and peripheral dopamine receptor agonist and they are antipsychotic agents. Antipsychotics have variable antagonist actions at dopaminergic, muscarinic, α -adrenoceptors and histaminergic receptors in brain and peripheral tissue (Charles and Robert, 1997) and probably the extract produced effect on dopaminergic pathway.

The tail suspension test is one of the most commonly used animal models for screening antidepressant compounds (Vogel, 2008). Many antidepressant compounds are able to reduce immobility as well as to promote the occurrence of escape related behaviour (Steru *et al.*, 1985). Antidepressant compounds significantly reduce immobility time in the tail suspended test in mice (Taqa, 2013). The observed decrease in the immobility time at lower dose of 87.5 mg/kg may lead to the conclusion that, ethanol extract of *T. globiferus* has little or no antidepressant activity in mice. However, the increase in the duration of immobility at higher doses may be due to sedative property of the extract. Luteolin from lemon has been reported to increase immobility time by inducing sedation and calming effect in mice (Terry *et al.*, 2009). However, compound that possess effective antidepressant activities significantly reduce the immobility time displayed by rodents after active and unsuccessful attempts to escape when suspended by the tail.

Pentylenetetrazole (PTZ)-induced seizure is a model used in screening agents that have antiepileptic property especially against petit mal epilepsy (Garba and Yaro, 2015). Agents that eradicate petit mal epilepsy act by enhancing GABA_A inhibitory action and block T-type Ca²⁺ current (Malawaska, 2005). Diazepam, sodium valproate and phenobarbitone are active antiepileptic drugs that produced their effects by enhancing

GABA-mediated inhibition in the brain (Porter *et al.*, 1984). PTZ has been shown to interfere with GABA neurotransmitter and the GABA receptor complex (Bum *et al.*, 2008). PTZ act as a selective antagonist to block the inhibitory effects at GABA_A receptors. GABA is the major inhibitory neurotransmitter in the brain (Rang *et al.*, 1998). The ethanol extract of *T. globiferus* at higher dose prolonged onset of acute seizure induced by PTZ insignificantly ($p \geq 0.05$) and offered minimum (16.67 and 33.33 %) protection against PTZ-induced seizure at tested doses. A study showed that prolongation of clonic seizure latency is an evidence of anticonvulsant activity (Olatokunboh *et al.*, 2009). Prolongation in onset of seizure in the PTZ model may provide evidence that at higher dose, the extract is effective in absence seizure. Sodium valproate, a standard drug offered 100 % protection against PTZ-induced seizure and is useful in the management of absence seizure (McNamara, 2006). The activity of the extract on PTZ may probably via GABAergic inhibitory mechanism.

Strychnine (STN) is a chemical convulsant which acts as competitive antagonist to block the inhibitory effects of glycine at all glycine receptors. Glycine is inhibitory neurotransmitter in the spinal cord (Rang *et al.*, 1998). Compounds that are effective in seizures induced by strychnine, act by enhancing the inhibitory action of glycine (Garba and Yaro, 2015). The ethanol extract of *T. globiferus* at tested doses (87.5, 175 and 350 mg/kg) offered no protection against strychnine-induced convulsion in mice. Therefore, the absence of anticonvulsant activity of the extract against STN-induced seizures suggests that the compounds may not interact with glycine receptor complex or glycine neurotransmission.

Maximal electroshock test (MEST) is used primarily for screening agents with activity against generalized tonic clonic seizure (Rang *et al.*, 1995), and is a model that evaluates the testing material's ability to protect against hind limb tonic extension (HLTE). Ability of the antiepileptic drugs to protect HLTE in MEST predicts its anticonvulsant activity which acts to prevent the spread of the epileptic seizure discharge from an epileptic focus during seizure (Raza *et al.*, 2001). Antiepileptic drugs such as carbamazepine, oxcarbazepine and lamotrigine suppress HLTE in MEST (Browning 1992). The extract offered no protection on chicks against Maximal electroshock and suggests that the extract is not effective in the management of generalized tonic clonic seizure.

Picrotoxin is a selective non-competitive antagonist of GABA being used in the chemical inductions of seizures by promoting chloride channel opening at GABA_A receptor (Meldum and Rogawski, 2007). The ethanol extract of *T. globiferus* produced a dose dependent protection against subcutaneous picrotoxin-induced convulsion in mice. The fact that the extract protected mice against subcutaneous picrotoxin-induced convulsion further confirmed that the plant extract contains a compound that facilitates GABA transmission. The effect produced by the extract may probably act through GABAergic inhibitory mechanism by blocking the effects of picrotoxin on the GABA receptors. This is also supported by the action on PTZ.

Interactive study was conducted using hole-board and ketamine-induced sleep test. Hole-board is an accepted model for evaluation of sedation condition (Goodman *et al.*, 2006) and the activity of the extract and fractions are more in the hole-board test. However, bicuculline and ketamine were used to evaluate the mechanism of action of

the fraction which was observed to show sedative activity and may produce this activity via GABA_A or NMDA receptor. Bicuculline is GABA_A receptor antagonist while ketamine is NMDA receptor antagonist. Bicuculline increased the number of head dips in mice by occupying GABA_A receptors but diazepam and EF neutralized the effects (Fig. 4.17), this showed that diazepam and EF may compete with bicuculline on GABA_A receptors. GABA_A receptor directly gates to Cl⁻ ionophore and has modulatory binding sites for benzodiazepine (Ritcher *et al.*, 2012). Like diazepam, EF may produce its effect by binding to GABA_A receptor. However, ketamine does not produce its effect via facilitation of GABA_A receptor, but produce its effect via antagonism of the excitatory neurotransmitter glutamic acid on the NMDA receptor (Katzung *et al.*, 2012). Ethylacetate fraction does not have effect on onset and duration of sleep induced by ketamine (Fig. 4.18) which showed that EF may not produce its effect via NMDA receptor. Reduction in the onset and prolong duration of sleep is an indication of CNS depression activity of extract or drug such as diazepam and ketamine (Chindo *et al.*, 2014). However, EF produced no effect in the onset and duration of sleep induced by ketamine.

The qualitative phytochemical screening conducted on the ethanol extract and fractions of *T. globiferus* revealed the presences of valuable chemical constituents, which might be responsible for the observed activities. Some chemical constituents such as alkaloids, flavonoids and saponins are known to have various pharmacological activities (Okeruku and Ani, 2001). Some investigators have attributed the sedative, anxiolytic and other pharmacological properties of a number of plants to their alkaloids, flavonoids, saponins, sterols and tannins constituents (Adzu *et al.*, 2002; Hosseinzadeh *et al.*, 2012; Edvaldo *et al.*, 2013).

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1 Summary

Tapinanthus globiferus commonly known as mistletoe (English), *kauchin kadanya* (Hausa), *eme-emi afomo* (Yaruba) and *okwuma osa* (Igbo) in Nigeria and belongs to the family of Loranthaceae. It is the most common mistletoe that grows on branches of *Vitellaria paradoxa* tree (host) in West Africa and used locally by traditional herbalist for the treatment of insomnia, epilepsy, anxiety, headaches and hypertension.

This study was investigated phytochemical constituents and evaluated the toxicity as well as some neuropharmacological properties of ethanol extract of *Tapinanthus globiferus* and its hexane, chloroform, ethylacetate and butanol fractions in Wister rats, Swiss Albino mice and chicks. These were carried out using standard methods such as Lorke method for acute toxicity testing, assays for sub-acute toxicity, diazepam- and ketamine-induced sleep, hole-board, beam-walk assay, elevated plus-maze, elevated staircase, open field, haloperidol-induced catalepsy, apomorphine-induced climbing, tail suspensions, maximal electroshock, pentylenetetrazole-, strychnine- and picrotoxin-induced convulsion. Experiments were in male and female mice and rats except MES which was in chicks, using oral, intraperitoneal and subcutaneous routes. Data were analysed using ANOVA followed by Dunnett's *post-hoc* test and data obtained over time was analysed using repeated measure ANOVA followed by Bonferroni *post-hoc* test with results expressed as Means \pm SEM, and $p \leq 0.05$ considered significant. The extract was fractionated using hexane, chloroform, ethylacetate and butanol.

The results of the study revealed that, the ethanol extract and fractions of *T. globiferus* contained valuable chemical constituents such as alkaloids, flavonoids, saponins, steroids, terpenoids and tannins which have sedative activity compared to diazepam used in diazepam-induced sleep, hole-board, beam-walk assay, elevated plus-maze, elevated staircase and open field tests in mice. The ethanol extract and fractions of *T. globiferus* were slightly toxic *i.p.* and practically non-toxic *p.o.* in mice and rats. The extract had no toxic effects on body weight, haematology, biochemistry and histology after daily oral treatment of rats at lower doses but showed toxicity on liver, kidney and spleen at higher dose. Potentiation of diazepam-induced sleep as well as reduction in mean number of head dips and increase in mean number of foot slips by the extract and fractions strongly suggest CNS depressant effect of *T. globiferus*. The extract also showed CNS depressant activity in open field test by decreasing the number of line crossed and rearing, and no anxiolytic activity as indicated in the elevated plus maze and elevated staircase tests in mice. Potentiation of catalepsy induced by haloperidol and increased immobility time in tail suspension tests in mice showed that, the extract does not have antidepressant activity and an indication of CNS depression activity which was supported by reduction in climbing behaviour induced by apomorphine. The extract produced no effects on maximal electroshock and strychnine-induced convulsion but active against pentylentetrazole- and picrotoxin-induced convulsion in mice. Ethylacetate fraction (EF) had no effect in ketamine-induced sleep but inhibit bicuculline activity in hole-board test in mice. EF may produce its effect through GABA_A receptor as observed in hole-board tests and ketamine-induced sleep using bicuculline (GABA_A receptor antagonist) and ketamine (NMDA receptor antagonist) respectively.

6.2 Conclusion

The major inference drawn from the above finding is in support for the use of *Tapinanthus globiferus* in traditional medicine in the treatment of neuropsychiatric (specifically, insomnia) and neurological (specifically, epilepsy) disorders and may produce its effect via GABA_A receptor. The extract of *T. globiferus* is relatively safe at lower doses. It can be concluded that *Tapinanthus globiferus* had good activity against sedation. However, it deserves further attention in the search for newer agents for the treatment of neuropsychiatric and neurological diseases.

6.3 Recommendations

- i. It is important to carry out column chromatography of *Tapinanthus globiferus* fractions with a view to purify and obtain a novel lead molecule for sedative activity.
- ii. More pharmacological assay techniques such as 5-HT binding assay, GABA_A binding assay, histamine H-receptor binding, dopamine D-receptor assay, and kindling assay should also be employed to establish the exact mechanism of action of the novel lead molecule.
- iii. Finally, chronic toxicity study should be conducted to ensure the overall safety of the new compound.

6.4 Contributions to Knowledge

This research made contributions to knowledge in the following areas:

- i. It identified the presence of alkaloids, anthraquinones, flavonoids, cardiac glycosides, saponins, steroids, terpenoids and tannins in the ethanol extract and fractions of *T. globiferus* which were found to be responsible for activity of the plant.
- ii. It determined the safety doses of the ethanol extract and fractions of *T. globiferus*, which in terms of LD₅₀ (in mg/kg) ranged from 1,270 to > 5,000 and 1,100 to 3,810 respectively.
- iii. It provided pharmacological evidence to back the use of *T. globiferus* in the management of insomnia based on its sedative-hypnotic (anxiolytic) properties as studied in diazepam-induced sleep, hole-board, beam-walk assay, elevated plus-maze, elevated staircase and open field tests in mice.
- iv. Study demonstrated that the ethylacetate fraction of *T. globiferus* is the most active CNS depressant among the fractions studied in diazepam-induced sleep, hole-board and beam-walk assay.
- v. It identified the mechanism of action of the most active fraction being the ethylacetate fraction as enhancing GABA activity studied in hole-board test.

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APPENDICES

Appendix I: Effect of Ethanol Extract of *T. globiferus* on Onset and Duration in Diazepam-induced Sleep in Mice

Treatment (mg/kg)	Onset of sleep (min.)	Duration of sleep (min.)
NS 10 ml/kg	17.33 ± 2.62	37.00 ± 19.50
TgE (87.5)	10.50 ± 0.22 ^a	85.50 ± 28.24
TgE (175)	7.83 ± 1.50 ^a	146.83 ± 26.03 ^a
TgE (350)	5.00 ± 1.00 ^a	170.83 ± 31.35 ^a

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, Diazepam (25mg/kg), route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline.

Appendix II: Effect of Ethylacetate Fraction on Onset and Duration in Diazepam-induced Sleep in Mice

Treatment (mg/kg)	Onset of sleep (min.)	Duration of sleep (min.)
NS 10 ml/kg	16.67 ± 2.32	52.00 ± 7.32
EF (75)	8.67 ± 1.09 ^a	53.33 ± 5.97
EF (150)	7.17 ± 0.83 ^a	110.33 ± 14.60 ^a
EF (300)	4.33 ± 0.33 ^a	122.33 ± 25.02 ^a

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM, Diazepam (25 mg/kg), route of administration = intraperitoneal, EF = Ethylacetate Fraction, NS = Normal saline.

Appendix III: Effect of Ethylacetate Insoluble Fraction on Onset and Duration in Diazepam-induced Sleep in Mice

Treatment (mg/kg)	Onset of sleep (min.)	Duration of sleep (min.)
NS 10 ml/kg	12.50 ± 2.28	65.67 ± 3.16
EIF (50)	11.67 ± 0.67	58.00 ± 6.51
EIF (100)	11.00 ± 1.90	59.33 ± 13.26
EIF (200)	9.67 ± 2.65	58.67 ± 14.23

No significant difference between control and treated groups, one way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, Diazepam (25 mg/kg), route of administration = intraperitoneal, EIF = Ethylacetate Insoluble Fraction, NS = Normal saline.

Appendix IV: Effect of Butanol Fraction on Onset and Duration in Diazepam-induced Sleep in Mice

Treatment (mg/kg)	Onset of sleep (min.)	Duration of sleep (min.)
NS 10 ml/kg	11.33 ± 1.12	53.00 ± 5.18
BF (250)	8.50 ± 1.61	55.67 ± 11.88
BF (500)	5.33 ± 0.84 ^a	110.33 ± 20.55 ^a
BF (1000)	5.83 ± 0.79 ^a	131.33 ± 20.65 ^a

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, Diazepam (25 mg/kg), route of administration = intraperitoneal, BF = Butanol Fraction, NS = Normal saline.

Appendix V: Effect of Residual Fraction on Onset and Duration in Diazepam-induced Sleep in Mice

Treatment (mg/kg)	Onset of sleep (min.)	Duration of sleep (min.)
NS 10 ml/kg	13.50 ± 2.17	117.67 ± 15.63
RF (250)	10.17 ± 1.45	124.00 ± 22.46
RF (500)	6.33 ± 1.20 ^a	124.33 ± 16.68
RF (1000)	5.33 ± 0.99 ^a	156.33 ± 13.54

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, Diazepam (25 mg/kg), route of administration = intraperitoneal, RF = Residual Fraction, NS = Normal saline.

Appendix VI: Comparative Effect of Fractions on Onset and Duration in Diazepam-induced Sleep in Mice

Treatment (mg/kg)	Onset of sleep (min.)	Duration of sleep (min.)
NS 10 ml/kg	11.83 ± 1.25	53.00 ± 5.48
EF (150)	7.00 ± 0.37 ^a	112.00 ± 14.75 ^a
EIF (200)	10.50 ± 2.23	58.00 ± 13.31
BF (500)	7.00 ± 0.26 ^a	108.33 ± 18.36 ^a
RF (500)	8.50 ± 0.99	50.83 ± 3.86

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, Diazepam (25 mg/kg), route of administration = intraperitoneal, EF = Ethylacetate Fraction, EIF = Ethylacetate Insoluble Fraction, BF = Butanol Fraction, RF = Residual Fraction, NS = Normal saline.

Appendix VII: Effect of Ethanol Extract of *T. globiferus* on Exploratory Activity Using Hole-board Test in Mice

Treatment (mg/kg)	Number of head dips
NS 10 ml/kg	18.67 ± 3.09
TgE (87.5)	8.33 ± 1.52 ^a
TgE (175)	9.33 ± 1.74 ^a
TgE (350)	9.33 ± 2.08 ^a
DZP (2)	9.50 ± 1.80 ^a

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

Appendix VIII: Effect of Ethylacetate Fraction on Exploratory Activity Using Hole-board Test in Mice

Treatment (mg/kg)	Mean no. of head dips
NS 10 ml/kg	14.33 ± 1.61
EF (75)	14.33 ± 2.04
EF (150)	11.50 ± 1.77
EF (300)	5.83 ± 2.74 ^a
DZP (2)	4.83 ± 2.12 ^a

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, EF = Ethylacetate Fraction, NS = Normal saline, DZP = Diazepam.

Appendix IX: Effect of Butanol Fraction on Exploratory Activity Using Hole-board Test in Mice

Treatment (mg/kg)	Mean no. of head dips
NS 10 ml/kg	16.17 ± 1.01
BF (250)	11.33 ± 1.82
BF (500)	9.83 ± 2.47
BF (1000)	8.33 ± 2.88
DZP (2)	7.83 ± 2.07 ^a

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, BF = Butanol Fraction, NS = Normal saline, DZP = Diazepam.

Appendix X: Comparative Effect of Fractions on Exploratory Activity Using Hole-board Test in Mice

Treatment (mg/kg)	Mean no. of head dips
NS 10 ml/kg	14.83 ± 1.35
EF (300)	5.33 ± 0.92 ^a
BF (1000)	7.00 ± 1.18
DZP (2)	5.67 ± 0.92 ^a

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, EF = Ethylacetate Fraction, BF = Butanol Fraction, NS = Normal saline, DZP = Diazepam.

Appendix XI: Effect of Ethanol Extract of *T. globiferus* on Number of Foot Slips and Time Spent in Beam Walk Test in Mice

Treatment (mg/kg)	Number of foot slips	Time spent on Beam (sec.)
NS 10 ml/kg	0.33 ± 0.33	7.00 ± 0.81
TgE (87.5)	1.17 ± 0.60	10.70 ± 1.88
TgE (175)	1.83 ± 0.60	10.77 ± 1.82
TgE (350)	3.50 ± 0.43 ^a	12.45 ± 1.25 ^a
DZP (1)	8.00 ± 0.26 ^a	15.02 ± 1.16 ^a

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

Appendix XII: Effect of Ethylacetate Fraction on Number of Foot Slips and Time Spent in Beam Walk Test in Mice

Treatment (mg/kg)	Mean no. of foot slips	Mean time spent (sec)
NS 10 ml/kg	0.67 ± 0.33	7.17 ± 0.75
EF (75)	0.83 ± 0.40	8.67 ± 1.52
EF (150)	1.50 ± 0.56	10.67 ± 1.20
EF (300)	3.50 ± 1.20 ^a	10.60 ± 1.34
DZP (1)	4.50 ± 0.43 ^a	11.83 ± 1.25 ^a

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, EF = Ethylacetate Fraction, NS = Normal saline, DZP = Diazepam.

Appendix XIII: Effect of Butanol Fraction on Number of Foot Slips and Time Spent in Beam Walk Test in Mice

Treatment (mg/kg)	Mean no. of foot slips	Mean time spent (sec)
NS 10 ml/kg	1.17 ± 0.40	6.50 ± 0.62
BF (250)	1.17 ± 0.48	7.67 ± 1.33
BF (500)	2.33 ± 0.88	9.50 ± 1.80
BF (1000)	2.50 ± 0.22	9.67 ± 1.31
DZP (1)	4.83 ± 0.31 ^a	11.83 ± 2.21

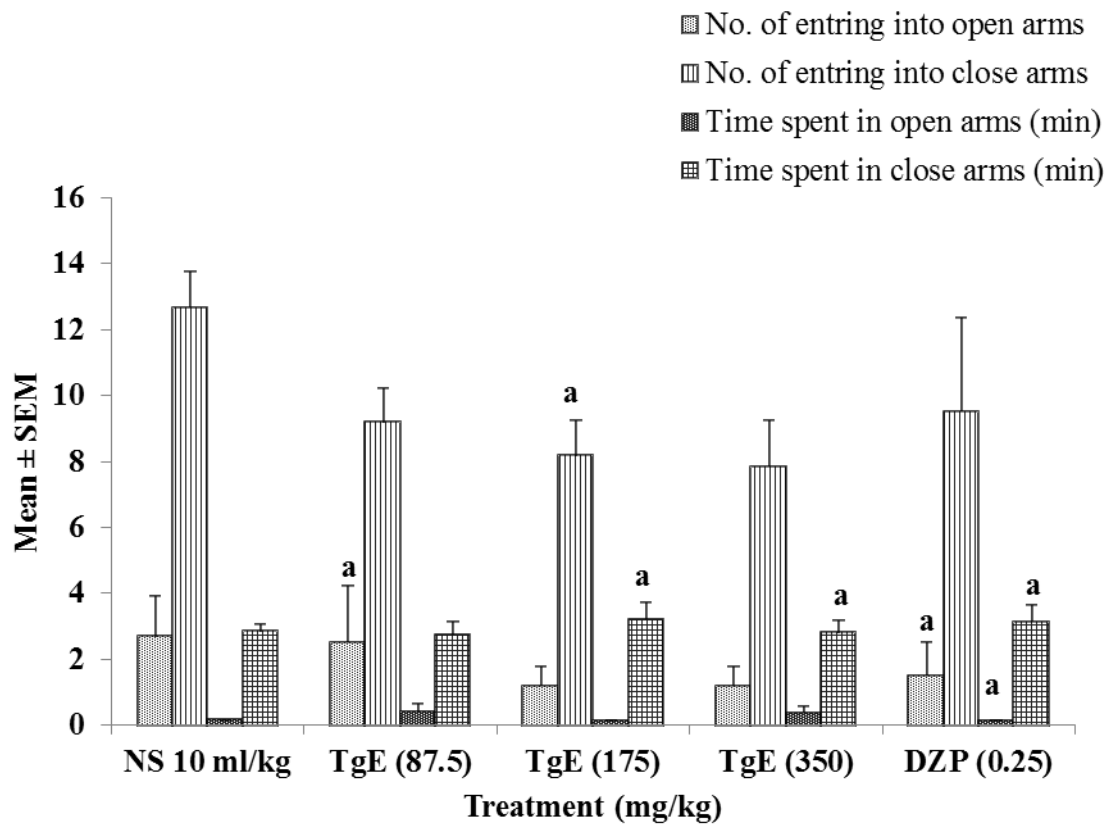
^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, BF = Butanol Fraction, NS = Normal saline, DZP = Diazepam.

Appendix XIV: Comparative Effect of Fractions on Number of Foot Slips and Time Spent in Beam Walk Test in Mice

Treatment (mg/kg)	Mean no. of foot slips	Mean time spent (sec)
NS 10 ml/kg	1.00 ± 0.37	4.83 ± 0.60
EF (300)	3.87 ± 0.61 ^a	9.67 ± 1.78 ^a
BF (1000)	3.23 ± 0.25 ^a	9.33 ± 1.05
DZP (1)	5.33 ± 0.49 ^a	9.83 ± 1.42 ^a

^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, EF = Ethylacetate Fraction, BF = Butanol Fraction, NS = Normal saline, DZP = Diazepam.

Appendix XV: Effect of Ethanol Extract of *T. globiferus* on Number of entries and Time Spent in Open and Close arms of Elevated Plus-maze Test in Mice



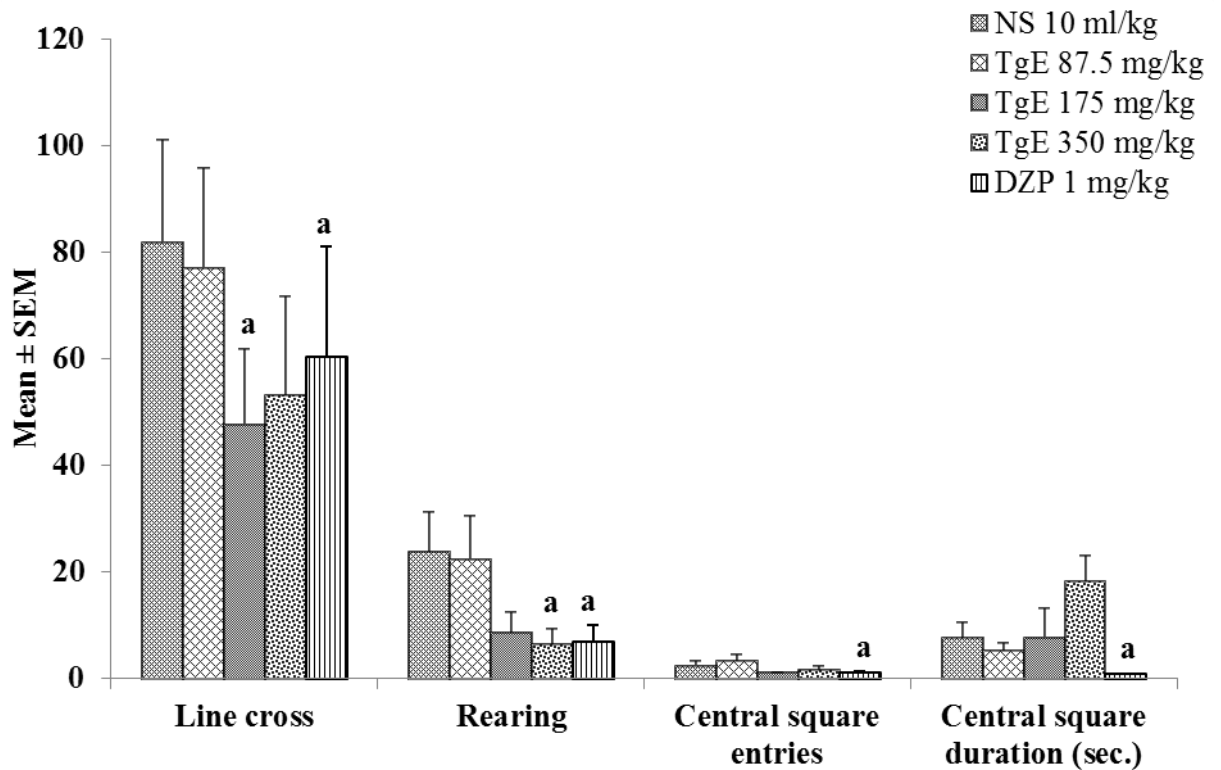
^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

Appendix XVI: Effect of Ethanol Extract of *T. globiferus* on Number of Steps Climbed and Number of Rearing in Staircase test in Mice

Treatment (mg/kg)	Number of steps climbed	Number of rearing
NS 10 ml/kg	27.17 ± 3.24	15.50 ± 2.78
TgE (87.5)	25.00 ± 2.80	14.33 ± 3.57
TgE (175)	24.17 ± 3.13	13.33 ± 3.42
TgE (350)	13.50 ± 2.09 ^a	5.83 ± 1.74
DZP (0.25)	11.83 ± 4.38 ^a	2.83 ± 2.29 ^a

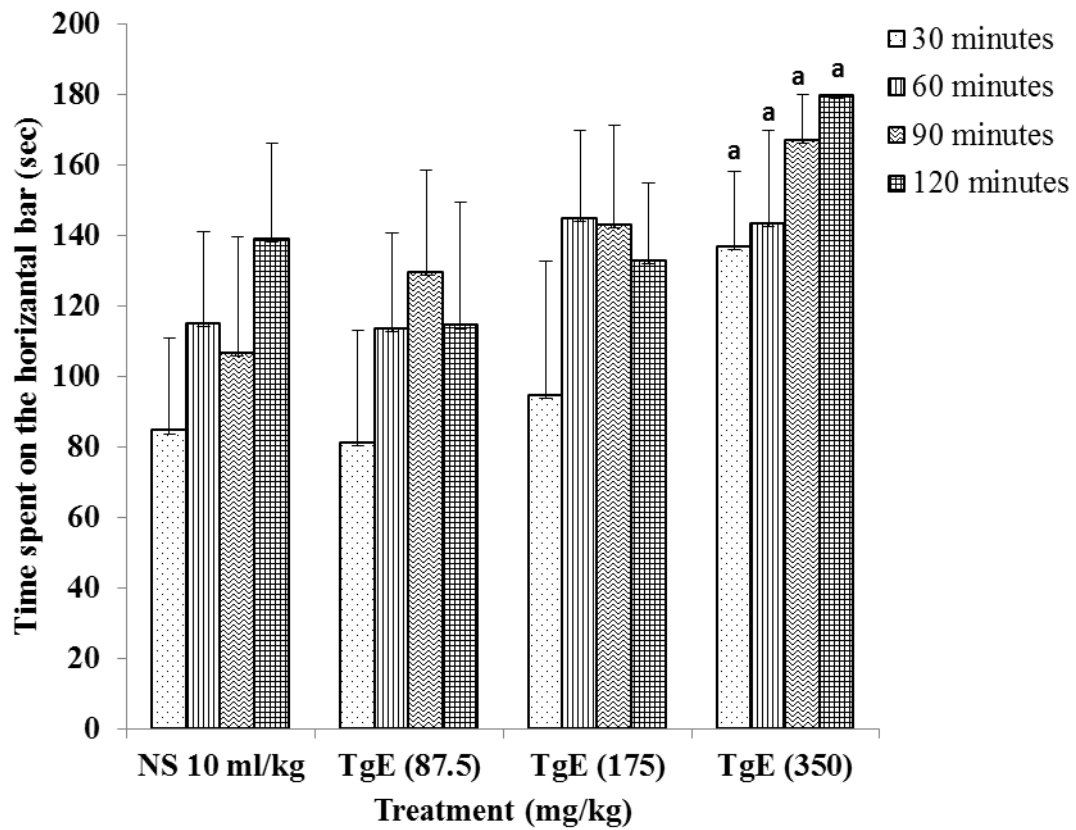
^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

Appendix XVII: Effect of Ethanol Extract of *T. globiferus* on Line cross, Number of Rearing, Central square entries and Central square duration in Open Field Test in Mice



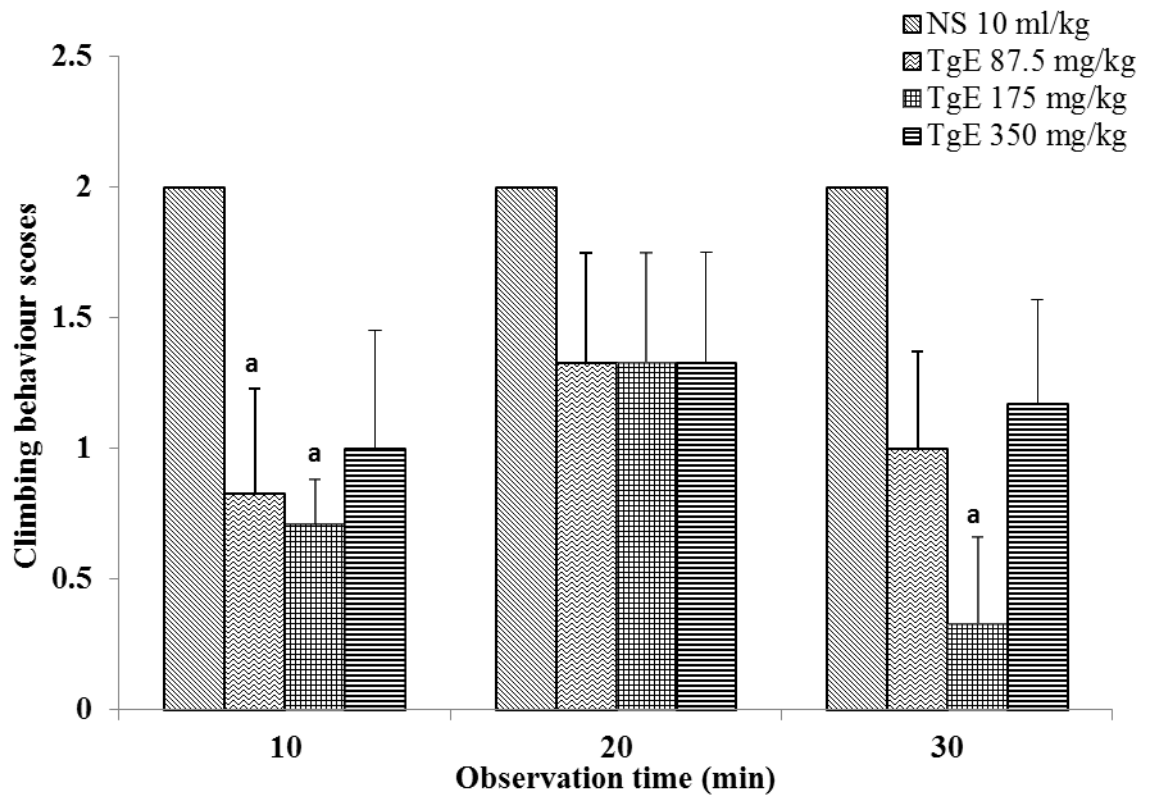
^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, DZP = Diazepam.

Appendix XVIII: Effect of Ethanol Extract of *T. globiferus* on Haloperidol-induced Catalepsy in Mice



^a $p \leq 0.05$ compared to NS and no significant difference over time—repeated measure ANOVA followed by Bonferroni *post-hoc* test. $n = 6$, Data = Mean \pm SEM, Haloperidol (1.0 mg/kg), route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline.

Appendix XIX: Effect of Ethanol Extract of *T. globiferus* on Apomorphine-induced Climbing Behaviour in Mice



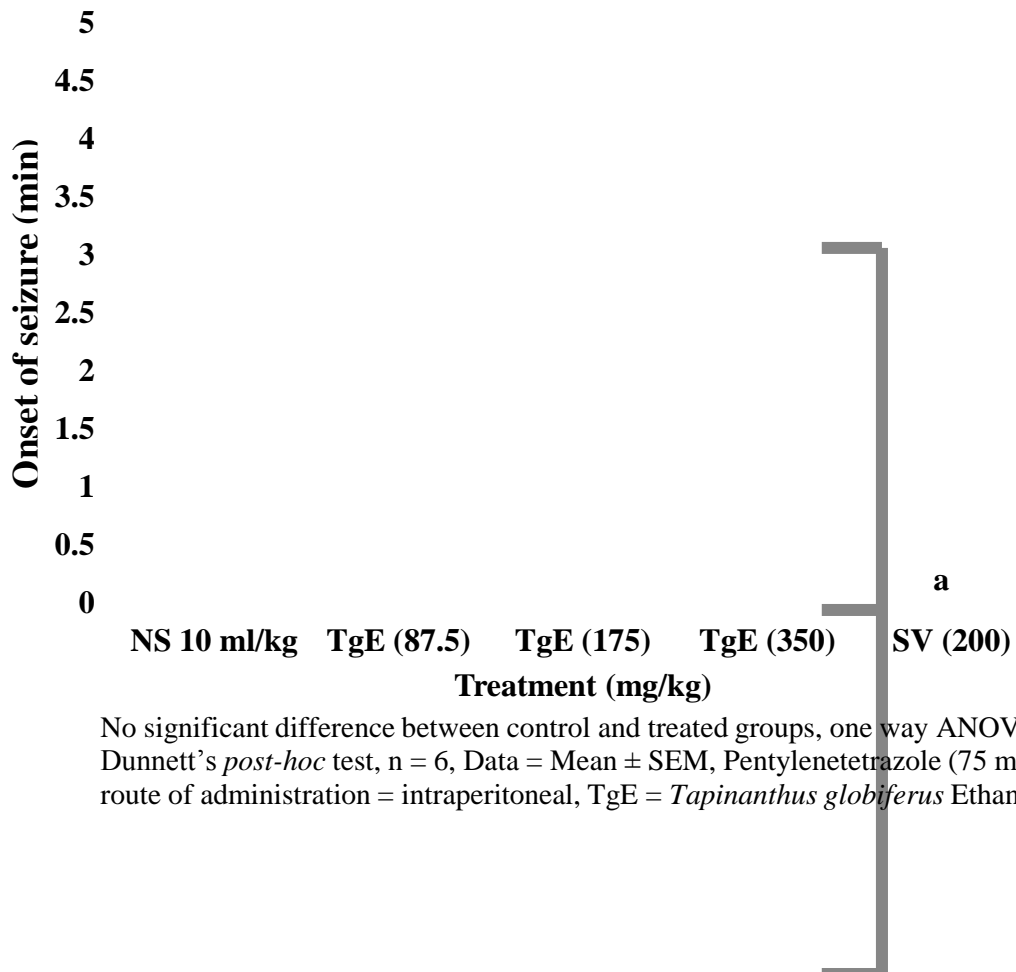
^a $p \leq 0.05$ compared to NS, non-parametric test; Kruskal-Wallis followed by Duni's test. $n = 6$, Data = Mean \pm SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, Apomorphine (3 mg/kg s.c).

Appendix XX: Effect of Ethanol Extract of *T. globiferus* on Immobility Time in Tail Suspension Test in Mice

Treatment (mg/kg)	Immobility (sec)
NS 10 ml/kg	123.17 ± 8.67
TgE (87.5)	119.50 ± 8.34
TgE (175)	161.83 ± 13.15
TgE (350)	162.17 ± 14.55
IMP (4)	35.33 ± 9.64 ^a

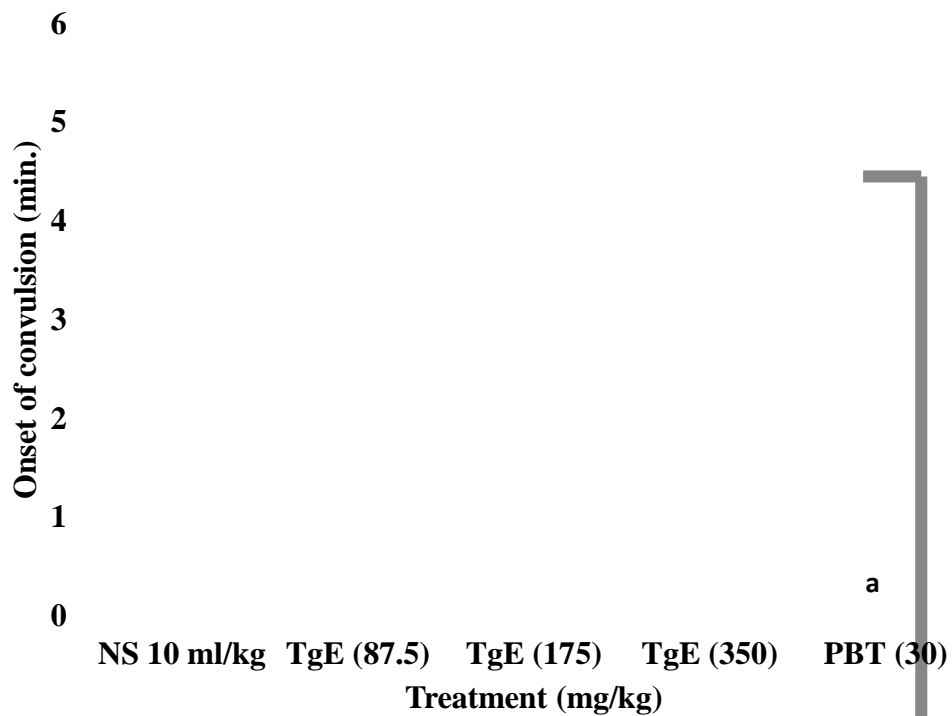
^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal saline, IMP = Imipramine.

Appendix XXI: Effect of Ethanol Extract of *T. globiferus* on Onset in Pentylenetetrazole-induced Convulsion in Mice



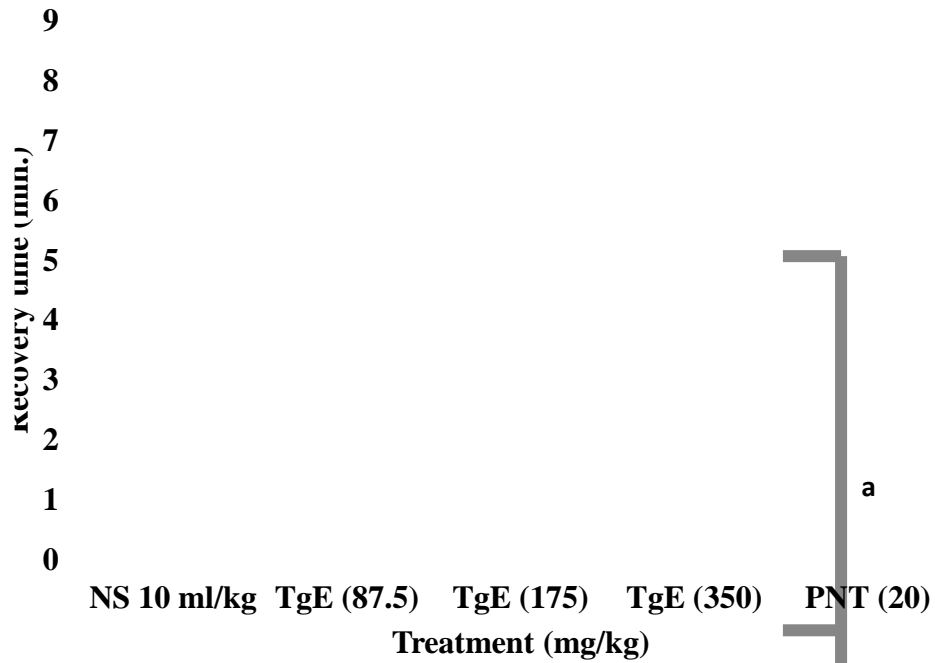
No significant difference between control and treated groups, one way ANOVA followed by Dunnett's *post-hoc* test, n = 6, Data = Mean ± SEM, Pentylenetetrazole (75 mg/kg route of administration = intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract)

Appendix XXII: Effect of Ethanol Extract of *T. globiferus* on Onset in Strychnine-induced Convulsion in Mice



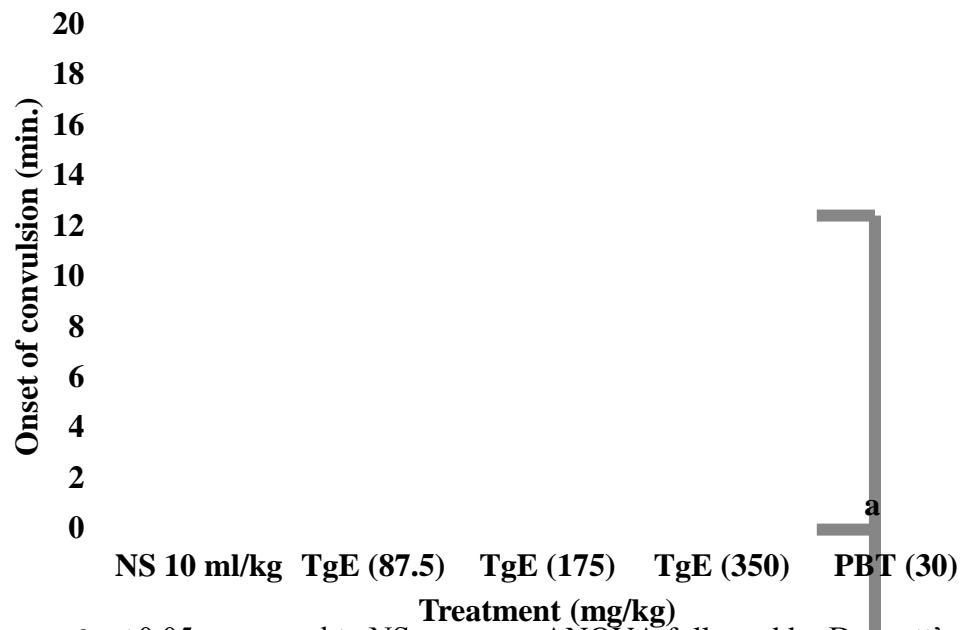
^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-hoc* test, $n = 6$, Data = Mean \pm SEM, Strychnine (1.2 mg/kg s.c), route of administration intraperitoneal, TgE = *Tapinanthus globiferus* Ethanol Extract, NS = Normal Saline

Appendix XXIII: Effect of Ethanol Extract of *T. globiferus* on Recovery Time
Recovery in Maximal Electroshock-induced Convulsion in
Chicks



^a $p \leq 0.05$ compared to NS, One way ANOVA followed by Dunnett's *post-ho*-10, Data = Mean \pm SEM, route of administration = intraperitoneal, TgE = *Tap globiferus* Ethanol Extract, NS = Normal saline, PNT = Phenytoin.

Appendix XXIV: Effect of Ethanol Extract of *T. globiferus* on Picrotoxin-induced Convulsion in Mice



^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnett's *post* hoc, Data = Mean \pm SEM, route of administration = intraperitoneal, TgE = *globiferus* Ethanol Extract, NS = Normal saline, PBT = Phenobarbitone,

Appendix XXV: Effect of Bicuculline, Diazepam and Ethylacetate Fraction of *T. globiferus* on Hole-board Test in mice

Treatment (mg/kg)	Mean no. of head dips
NS 10 ml/kg	16.17 ± 1.11
BCL (5) + NS 10 ml/kg	23.83 ± 2.00 ^a
DZP (2)	4.83 ± 0.87 ^a
BCL (5) + DZP (2)	15.50 ± 1.52
EF (300)	5.83 ± 1.68 ^a
BCL (5) + EF (300)	15.83 ± 1.08

^a $p \leq 0.05$ compared to NS, one way ANOVA followed by Dunnet's *post-hoc* test. n = 6, Data = Mean ± SEM, Drug administration = intraperitoneal, BCL = Bicuculline, DZP = Diazepam, EF = Ethylacetate Fraction, NS = Normal saline.

Appendix XXVI: Effect of Ethylacetate Fraction on Onset and Duration in Ketamine-induced Sleep in Mice

Treatment (mg/kg)	Onset of sleep (min.)	Duration of sleep (min.)
NS 10 ml/kg	2.00 ± 0.26	14.00 ± 2.03
EF (75)	1.83 ± 0.31	24.50 ± 5.32
EF (150)	1.83 ± 0.31	17.17 ± 4.70
EF (300)	1.50 ± 0.22	24.00 ± 3.44

No significant difference between control and treated groups, One way ANOVA followed by Dunnett's *post-hoc* test. n = 6, Data = Mean ± SEM, Ketamine (100 mg/kg), route of administration = intraperitoneal, EF = Ethylacetate Fraction, NS = Normal saline.

Appendix XXVII: Publications from the Thesis

1. **Abdullahi, M.H.**, Danjuma, N.M., Aliyu, M. and Abubakar, S. (2015). Effects of crude ethanol extract of *Tapinanthus globiferus* A. Rich on functional and structural integrity of the rat kidney. *International Journal of Herbs and Pharmacological Research*, **4(3)**: 33 – 39.
2. **Abdullahi, M.H.**, Danjuma, N.M., Yaro, A.H. and Abubakar, S. (2015). Effects of oral administration of ethanolic extract of *Tapinanthus globiferus* A. Rich on liver functional in rats. *Bayero Journal of Pure and Applied Sciences*, **8(2)**: 129 – 134.
3. **Abdullahi MH**, Kwanashie HO, Danjuma NM, Musa AM (2017). Phytochemical, Haematological and Histopathological Evaluation of the Ethanol Extract of *Tapinanthus Globiferus* A. Rich (Loranthaceae) in Rats. *Bayero Journal of Biomedical Sciences*, **2(2)**: 230-237.
4. **Mustapha H. Abdullahi**, Helen O. Kwanashie, Nuhu M. Danjuma and Aliyu M. Musa (2018). Sedative and Anticonvulsant Evaluation of *Tapinanthus Globiferus* A. Rich (Loranthaceae) in Mice and Chicks. *Journal of Pharmacy and Bioresources*, **15(1)**: 70 - 77.

Appendix XXVIII: Conferences Presentations from the Thesis

1. **Abdullahi, M.H.,** Kwanashie, H.O. and Danjuma, N.M. Sedative and Anticonvulsant Properties of *Tapinanthus Globiferus* A. Rich (Loranthaceae) in Mice and Chicks. 14th Annual Conference and General meeting of the Neuroscience Society of Nigeria (NSN), School of Postgraduate Studies Auditorium, ABU, Zaria (1st - 4th August, 2016).
2. **Abdullahi MH,** Kwanashie HO, Danjuma NM, Musa AM (2017). Phytochemical, Haematological and Histopathological Evaluation of the Ethanol Extract of *Tapinanthus Globiferus* A. Rich (Loranthaceae) in Rats. 5th Scientific Conference and Workshop of the Nigerian Association of Pharmacists in Academia (NAPA), Faculty of Pharmaceutical Sciences, A.B.U. Zaria (18th – 21st April, 2017).