SOME NEUROPHARMACOLOGICAL STUDIES ON THE STEM BARK OF *RANDIA NILOTICA* STAPF. (RUBIACEAE)

BY

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A DISSERTATION SUBMITTED TO THE POSTGRADUATE SCHOOL AHMADU BELLO UNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIRMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PHARMACOLOGY

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NOVEMBER, 2007

DECLARATION

I declare that the work in the dissertation entitled "some neuropharmacological studies on the stem bark of *Randia nilotica* stapf. (rubiaceae)" has been performed by me in the department of pharmacology and clinical pharmacy under the supervision of Prof. I. Abdu-Aguye, Dr. J. A. Anuka and Prof. I.M. Hussaini.

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

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Name of Student	Signature	Date

CERTIFICATION

This dissertation entitled "SOME NEUROPHARMACOLOGICAL STUDIES ON THE STEM BARK OF *RANDIA NILOTICA* STAPF. (RUBIACEAE)" by Danjuma, Nuhu Mohammed meets the regulations governing the award of the degree of Doctor of Philosophy of 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 the memory of my late mother Hafsat Umar.

ACKNOWLEDGEMENT

I wish to acknowledge and express my gratitude to my supervisors, Prof. I. Abdu-Aguye, Dr. J. A. Anuka and Prof. I. M. Hussaini for their various contributions in making this work a success. I wish to acknowledge also, the constant words of encouragement from Prof. E. M. Abdurrahman, Vice Chancellor Kaduna State University and the assistance of Dr. O. A. Salawu, Deputy Director, National Institute of Pharmaceutical Research and Development Abuja.

My gratitude also goes to my colleagues and friends for their advice, assistance and encouragement: Drs. A. U. Zezi, A. A. Ahmadu, B. A. Chindo, Malls. A. H. Yaro, A.M. Musa, A. Abubakar, I. Adamu, M. G. Magaji, S. U. Gallah, M. Nuhu, M. A. Abdullahi Mrs. B. B. Maiha (HOD. Pharmacology), Mr. C. Ayewah (Late), and others too numerous to mention.

I am also grateful to my family particularly my father Alh. Danjuma Muhammad, wife Halima Muhammad and Uncle M.K. Falalu for understanding and moral support. Furthermore, the final part of 'this dissertation has been completed with support from the ABU MacArthur Foundation Projects Grant'.

May Allah bless all of you abundantly. Thank you.

ABSTRACT

The commonest practical use of behavioural tests is probably in the search for new drugs. Medicinal plants have been used in the development of new drugs and continue to play an invaluable role in the drug discovery process. These plants/herbs are relatively cheap and available and their use depends on ancestral experience. The majority of the population in developing countries remain dependent on them for healthcare. *Randia nilotica* stapf is a lowland shrub or tree widespread in the Sudan and reported from lowland habitats in Central and East Africa as well as Cameroon and Nigeria. In ethnomedicine, a decoction is used orally for treatment of mental breakdown and for convulsions. It is also used against epilepsy and madness. The main objective of this project is to establish scientific basis for the use of *Randia nilotica* in traditional medicine for the treatment of neurodegenerative and neuropsychiatric illnesses. It also seeks to determine active chemical constituents of *Randia nilotica* as well as its acute toxicity profile.

The test systems employed for primary screening of *Randia nilotica* included Lorke method (1983) of acute toxicity determination, diazepam induced sleep, hole board test, pentobarbitone induced sleep, performance on treadmills (rota rod), mouse beam walk assay and amphetamine induced stereotype behaviour test. Others were electroshock induced convulsion test, pentylenetetrazole induced convulsion test, strychnine induced convulsion test as well as forced swim test in rats and tail suspension test in mice. In addition, electro-encephalographic studies in rats was conducted. In these experiments, rats (180-250g), Swiss albino mice (18 –25g) and black ranger cockerels (1 day old) were used where appropriate. The diazepam induced sleep method was used to elucidate the more potent central nervous system active morphological parts of *Randia nilotica* (leaf, stem bark, root bark) as well as its fractions (saponins, flavonoids, aqueous butanolic residue) and sub-fractions (SF_1-SF_5). Data

generated was analysed using t-test and significant difference determined using one-way analysis of variance (ANOVA) followed by Dunnet's and Scheffe's post hoc tests. Results were expressed as mean ±standard error of the mean (±SEM). Values that are ≤ 0.05 were considered significant.

Results of studies carried out revealed high potency for stem bark extract compared to leaf or root bark extracts. This is evident in the increase in duration of sleep as observed in diazepam sleep test which was significant ($p \leq 0.001$). It increased from 38.2±5.78 min. (Normal Saline) to 165.8±22.9 min. (stem bark), 113.5±12.8min. (leaf) and 64±13.0min. (root bark). Moreover, the activity of the stem bark was observed at 20mg/kg dose compared to 200mg/kg (10 times higher) for the leaf extract. Similarly, the saponin fraction exhibited higher potency compared to flavonoid or aq. butanolic residue of the stem bark extract. Sleeping time was increased significantly ($p \leq 0.05$) compared with normal saline i.e from 38.2±6min to 117±20.8min. The flavonoid and water fractions also showed significant increases ($p \leq 0.005$) in sleeping time to 68.6±11min and 77.0±10.0min. respectively compared to normal saline. These increases in sleep duration were observed with the extracts at 20mg/kg (flavonoid, water fraction) and 2mg/kg (saponin fraction). This indicated that the saponin fraction is ten times more potent than either flavonoid or aqueous fractions. SF_1 showed least decrease in onset and highest increase in sleep duration followed by SF_4 and then SF_2. The LD_{50} values determined by intraperitoneal administration in mice for stem bark, root bark and leaf extracts were 282.2mg/kg, 282.2mg/kg and 2154.1mg/kg respectively. Those of the saponin, flavonoid, aqueous fraction were 11.1mg/kg, 471.2mg/kg and 471.2mg/kg respectively.

In the hole board experiment, the hydroalcoholic stem bark extract showed a dose dependent decrease in number of head dips which was significant ($p \leq 0.05$) at 20mg/kg body weight from 7.7±2.5 mean number of head dips (normal saline) to 1.3±0.8. Similarly, the saponin fraction showed a significant ($p \leq 0.05$) dose dependent decrease in mean number of head dips in this experiment from 7.7±2.5 (normal saline) to 2.0±0.6 (5mg/kg), 1.2±0.4 (1.0mg/kg), 0.3±0.0 (2.0mg/kg) and 2.2±1.7 (Diazepam, 1mg/kg).

The stem bark extract did not produce any significant observable effect on motor coordination as determined in the rota rod test. Similarly, its saponin fraction did not produce any observable effect on motor coordination using the rota rod test.

In the Beam walk assay, significant difference ($p \leq 0.001$) was observed in mean number of foot slips between stem bark extracts of *Randia nilotica* (5, 10, 20mg/kg body weight), normal saline and Diazepam (control, 1.5mg/kg). The least number of foot slips was observed with normal saline (0.4±0.2) which subsequently increased in diazepam treated mice (10.8±1.9). Diazepam also showed a significant ($p \leq 0.001$) increase in number of foot slips compared to normal saline and extracts at 0.5, 1.0 and 2.0mg/kg (1.4±0.6, 1.8±0.7, 1.4±0.7 respectively). No difference was however observed in time to reach goal box between controls and extracts at all doses tested.

A biphasic effect was observed with stem bark extracts of *Randia nilotica* (5, 10, 20mg/kg) on amphetamine-induced stereotyped behaviour in mice. There was a significant ($p \leq 0.001$) reduction in jumping/climbing episodes produced by the extract from 937.0±22.3 (normal saline) to 103.2±31.2 (5mg/kg), 33.0±11.7 (10mg/kg), 69.8±13.8 (20mg/kg) and 13.0±5.0 (Chlorpromazine, 2mg/kg). Reduction in sniffing was also observed with extract at 5mg/kg

(83.4±18.6), which is significant ($p \leq 0.05$) compared with normal saline (1308.8±13.2). Higher doses paradoxically produced increases rather than decreases in sniffing. The extract (20mg/kg) attenuated mean count in limb licking from 201.8±15.5 (Normal Saline) to 55.6±19.5. Lower doses produced increases in limb licking.

The results of the anticunvulsant tests were also mixed. In the maximal electroshock test. The stem bark extract of *Randia nilotica* protected chicks against hind limb tonic extension (HLTE) by 90% at 20mg/kg comparable to phenobarbitone (PBT) with 90% protection as well. Both 5mg/kg and 10mg/kg produced a 50% inhibition. The saponin fraction on the other hand, protected mice against HLTE by 50% at 2mg/kg body weight. 0.5 and 1.0mg/kg of the saponins showed only a 20% inhibition compared with PBT (20mg/kg) which sowed 80% protection. On the other hand, the stem bark and saponin extracts did not show any effect on pentylenetetrazole (sc-PTZ) induced and strychnine induced seizure tests in mice. However, the mean number of myoclonic body twitches was significantly ($p \leq 0.05$) reduced by the stem bark extract in the sc-PTZ induced seizure test. 5mg/kg produced a 161% reduction while 10 and 20mg/kg produced 250% and 265% reduction respectively.

In the tail suspension test in mice, the HA stem bark extract of *Randia nilotica* showed no effect on duration of immobility time in mice at the doses tested. But some dose dependent decrease in immobility time was observed in the forced swim test in rats. The reduction was however not significant when compared to control. The saponins on the other hand showed a significant ($p \leq 0.05$) decrease in immobility time at 2.5mg/kg (82.4 ±7.9) and 10mg/kg (80.0±20.7) compared with normal saline (108.6±24.2).

Desynchronisation in the rat electroencephalograph (EEG) of the hyperstriatum (HS), optic tectum (OT) and pontine reticular formation (RF) was observed with the stem bark extract at 20mg/kg and 40mg/kg. A slight increase in muscle activity was also observed. At 80mg/kg however, there was synchronisation of HS, RF and OT while a reduction in electromyographic (EMG) activity was observed. The saponin fraction of *Randia nilotica* (2.5mg/kg) slightly synchronized EEG of the HS, OT and RF but with weak activation in EMG of the rat. At 5mg/kg and 10mg/kg body weight, synchronization of the HS, RF and OT was observed as well as a decrease in EMG activity throughout the period of observation.

These results suggested significant sedative effects of the stem bark extract of *Randia nilotica*, its saponin fraction and subfraction (SF_1). This may lend some scientific support for the uses of the plant in traditional medicinal practice in the treatment of neuropsychiatric and neurological diseases. It may also be suggested that the extracts are relatively safe at the dose levels tested. It can be concluded therefore that *Randia nilotica* merits further attention in the search for a new compound against neurodegenerative and neuropsychiatric disorders.

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ABBREVIATIONS AND SYMBOLS

The following abbreviations and symbols were used in the course of writing this project report:

α	Alpha
β	Beta
γ	gamma
5-HT	5-hydroxytryptamine
Ach	Acetylcholine
ADH	Antidiuretic hormone
AE	Adverse Effects
AFDs	Antiepileptic drugs
ANOVA	Analysis of variance
APA	American Psychiatric Association
BC	Before Christ
BZ_1	Alpha subunit of GABA receptor
BZ_2	Gamma subunit of GABA receptor
BZD	Benzodiazepine
Ca^{2+}	Calcium ions
CBZ	Carbamazepine
CM	cerebral malarice
Cm	centimeter
CNS	Central Nervous System
Comp.	Company

CPZ	Chlorpromazine
CYP3A4, CYP2D6	Isoenzymes of cytochrome enzymes
D2,DA2	Dopamine sub receptor type 2
DSM-IV	Diagnostic and statistical manual of mental disorders-fourth edition
DZP	Diazepam
EEG	Electroencephalography
EMG	Electromyograph
EPS	Extrapyramidal symptoms
ETX	Ethosuximide
G	grammes
GABA	Gamma amino butyric acid
GAD	Generalised anxiety disorder
GI	Gastrointestinal
H.S	Hyperstriatum
HA	Hydroalcoholic
HLTE	Hind Limb Tonic Extension
Hrs	hours
i.p	intraperitoneal
IMP	Imipramine
Kg	Kilogram
LD_{50}	Lethal dose or dose that can kill 50% of an animal population
MAO	Monoamine oxidase enzyme
MAOA	Monoamine oxidase type A receptors
MAOB	Monoamine oxidase type B receptors
MAOIs	Monoamine oxidase inhibitors

MEST	Maximum electroshock test
Mg	Milligram
Mins.	Minutes
Ml	milliliters
Mm	millimeter
NaSSA	Noradrenergic and specific serotonergic antidepressants
NE	Norepinephrine
NIH	National Institute of Health
NMDA	N-methyl-D-aspartate
NO	Nitric oxide
NOS	Nitric oxide synthase
O.T.	optic tectum
OCD	obsessive compulsive disorder
PBT	Phenobarbitone
PCP	Phencyclidine
PD	Panic disorder
PEMA	Phenylethylmalonamide
PIP	Phosphatidylinositol biphosphate
PTSD	post traumatic stress Disorder
PTZ	Pentylene tetrazole
QT-interval	Segment of electrocardiograph
R.F	Reticular formation
REM	Rapid eye movemet
RNBE	Stem bark extract of *Randia nilotica*
RNLE	Leaf extract of *Randia nilotica*

RNRE	Root bark extract of *Randia nilotica*
S.E.M	Standard error of the mean
SB	Stem bark
scPTZ	subcutaneous pentylene tetrazole
Sc-STN	subataneous injection of strychnine
SF_{1-5}	Sub-fractions 1-5 of the saponin fraction of *Randia nilotica*
SFRN	Saponin fraction of *Randia nilotica*
SNRIs	Selective serotonin/norepinephrine reuptake inhibitors
SP	Social Phobia
SSRIs	Selective serotonin re-uptake inhibitors
STN	Strychnine
TCAs	Tricyclic antidepressants
TLC	Thin Layer chromatography
TMP	traditional medical practitioners
USA	United State of America
USD	United states Dollars
VPA	Valproic acid
WFRN	aqueous butanolic residue of *Randia nilotica*
WHO	World Health Organization
WOES	weight gain, orthostatic hypotension, edema and sexual dysfunction

CHAPTER ONE

1.0 INTRODUCTION

Men ought to know that from the brain, and from the brain only, arise our pleasures, joys, laughter and jests, as well as our sorrows, pains, griefs and tears. Through it …..we think, see, hear…….. Hippocratis (5th Century B.C.)

1.1 STATEMENT OF RESEARCH PROBLEM

Medicinal plants have been used in the development of new drugs and continue to play an invaluable role in the drug discovery process (Cragg *et al.,* 1997; Farnsworth, 1994). Medicinal plant is any plant that contains substance(s) which can be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Sofowora, 1993). Traditional medical practitioners (TMP) use plants to cure various disease conditions. The World Health Organisation (WHO) has shown that, over 80% of the population in sub-saharan Africa (Mozambique) patronise TMPs (WHO- EML, 2007).

According to Meldrum (1997), plant extracts can be an important source of natural and safer drugs for the treatment of epilepsy. Extracts, fractions and pure compounds from several medicinal plants have been used in the treatment of epilepsy and have demonstrated anticonvulsant properties that need to be further investigated (Raza *et al.,* 1999). Similarly, several medicinal plants are known to have a great potential in the treatment of other neurological and neuropsychiatric diseases. Quite a number of such plants have been identified, particularly in Africa. Scientific bases for the use of most of the plants are not known. Hence there is the need to intensify research into these local medicinal plants so as to confirm their therapeutic values and establish scientific bases for their uses so as to prepare

them in a suitable dosage form for therapeutic uses and or provide novel lead molecules or precursors for the pharmaceutical industry.

Fortunately, Nigeria is blessed with vast varieties of plants that could be studied. One such plant is *Randia nilotica* stapf. Synomymous Latin binomials are *Lachnosiphonium niloticum* Hochst; *xeromphis nilotica* Raf.Sylva Tellur;*Catunaregam niloticum*.It is a lowland shrub or tree,widespread in Northern Nigeria and reported from lowland habitats in Central and East Africa as well as Cameroun and the Sudan (Lemmich *et al.*,1995).The plant is commonly known as 'Shagart-elmarfaein' in the Sudan, while in Northern Nigeria it is known as 'Barbaji','tsibra' or 'kwanarya'(Hausa).The Fulanis call it 'gial-goti'(Dalziel,1937).Branches of the plant are whitish, pubescent, armed with sharp spines 1-3 cm. long. Leaves and flowers are on much abbreviated lateral branchlets, borne in pairs just below the spines.The fruits are ellipsoid, 1.8-2.0 cm. long, 1.0-1.5 cm.broad, glabrous and two-celled with small black seeds (Hutchinson and Dalziel, 1963). Phytochemical screening of the stem bark revealed the presence of alkaloids, flavonoids, sterols and or triterpenes (El-kheir and Salih, 1980). Parts of *randia nilotica* have several uses in folk medicine. The stem bark, root bark and fruits from *Randia nilotica* are among the most potent materials used by the natives. A decoction is used orally for mental breakdown and for convulsions (Chhabra *et al.*, 1991). In Tanzania, the dried root is used against epilepsy and for treatment of madness (Hedberg *et al.*, 1983). In some parts of East Africa, pounded and macerated fresh fruit is used as a fish poison and as an emetic (Hedberg *et al.*, 1983; Dalziel, 1937). These claims hither-to, were not scientifically investigated.

The economic crisis through which developing countries are passing, has made it mandatory for them to seek cheaper ways of developing drugs from natural sources. Such plants as *Randia nilotica* could serve as raw materials for our local pharmaceutical industries. They

could also serve as a substitute for ingredients being imported at high costs, thus conserving our much needed foreign exchange earnings or better still as a veritable source of foreign exchange for the country.

Furthermore, drug development is now almost exclusively a business-driven activity. The world market by pharmaceutical sales amounts to almost 250 billion United states Dollars (USD) and this market is expected to grow with an average rate of 7 – 8‰ per year (Van Brummelen, 2001). The U. S. has about 40% of the world market, Europe about 25% and Japan 15%. Again there are currently 30 drugs that sell more than 1 billion USD per year. The best selling drugs belong to the following therapeutic areas: cardiovascular (hypertension, angina pectoris, heart failure), **central nervous system (Depression, Psychosis, Migraine),** Gastroenterology ((Ulcers, gastroesophageal reflux) and infectious diseases. Of the 30 top selling drugs in 1998, only three are biotechnology compounds, whereas all others are traditional drugs (Van Brummelen, 2001).

1.2 JUSTIFICATION FOR THE STUDY

The nervous and the endocrine systems, provide most of the control functions for the body (Guyton, 1982). In general, the nervous system controls the rapid activities of the body, such as muscular contractions, rapidly changing visceral events and even the rate of secretion of some endocrine glands.The brainstem, for example provides many special control functions, such as: control of respiration, cardiovascular system, gastrointestinal functions, many stereotyped movements of the body, equilibrium and control of eye movement (Guyton and Hall, 2000). The nervous system is unique in the vast complexity of the control action. It receives literally thousands of bits of information from different sensory organs, then

integrates all of them to determine the response to be made by the body. In other words, the information is processed in such a way that appropriate motor response occur.

These informations are coded in the form of neurotransmitters. There are over forty known neurotransmitters today (Rang *et al*., 1995). These include amino acids- glycine, glutamate, gammaminobutyric acid (GABA), acetylcholine; monoamines– dopamine, norepinephrine, 5-hydroxytryptamine; peptides– opiod peptides (enkephalins, endorphins etc), substance P, somatostatin, cholecystokinin; nitric oxide; endocannabinoids and a host of others (Nicoll, 2004).Some of the neurotransmitters are excitatory at effector organs(dopamine e.t.c) while others are inhibitory (GABA, Glycine). In most cases, a pair of the neurotransmitter exists and act in a complimentary manner to one another. Thus, when the activity of one system supplying an organ increases, the activity of the other usually decreases, thereby creating a balance.

Only a small fraction of the important sensory information causes an immediate motor response. Much of the remainder is stored for future control of motor activities and for use in the thinking processes. Most of this storage occurs in the cerebral cortex, but not all. Even the basal regions of the brain and perhaps even the spinal cord can store small amounts of information. This storage is the process known as memory (Guyton and Hall, 2000).

Most of what is called the subconscious activities of the body is controlled in the lower areas of the brain – the medulla, pons, mesencephalon, hypothalamus, thalamus, cerebellum and basal ganglia. Subconscious control of arterial blood pressure and respiration is achieved primarily in the reticular substance of the medulla and pons. Control of equilibrium is a combined function of the older portions of the cerebellum and the reticular substance of the

medulla, pons and mesencephalon. The co-ordinated turning movements of the head, of the entire body and the eyes are controlled by specific centres located in the mesencephalon, paleocerebellum and lower basal ganglia. Feeding reflexes, such as salivation in response to taste of food and licking of the lips, are controlled by areas in the medulla, pons, mesencephalon, amygdala and hypothalamus. Other activities such as sleep, wakefullness, extreme excitement and even different levels of mood such as exhilaration, depression and fear are controlled in higher centres of the brain – the cerebral cortex.

It is expected therefore from the foregoing, that changes in physiology and or anatomy of the brain will lead to serious disorders with attendant morbidity and mortality. These disorders are either neuropsychiatric or neurological.

Neuropsychiatric disorders are those that involve impairment in mood and cognition. Examples include anxiety disorders, mood disorders (depression, mania), schizophrenia, sleep disorders (insomnia etc), attention deficit hyperactivity disorders, obesity, eating disorders and several others. On the other hand, neurological disorders are associated generally with impairment in motor function. Examples are parkinsonism, seizure disorders (epilepsy), headache (migraine etc), stroke, alzheimers disease amongst others.

The prevalent rates for these disorders are varied. Depression which results from functional deficiency of monoaminergic (noradrenaline and or serotonin) transmission in the central nervous system (Rang *et al*, 1993) afflicts 14 million adults each year in the United State of America USA (Howland and Mycek, 2006). Its lifetime prevalent rate include 16% of adults (21% women, 13% men). The symptoms of depression are often subtle and unrecognized

both by patients and by physicians (William *et al*, 2006) which suggests a higher prevalence rate in less developed countries.

Similarly, anxiety (an unpleasant feeling of apprehension or fearful concern) has a life time prevalence rate of 24.9‰ in the US (Kessler *et al*, 1994). Post traumatic stress disorder (PTSD) which is a form of anxiety disorder has a life time prevalence rate of about 1% in the general population. Traumatised populations such as refugees can have a lifetime prevalence rate of 30 – 50% and a current rate of 10-38% (Tomb, 1994; Marshal *et al*., 1996). This might be the most common anxiety disorder in Africa. On the other hand, the total lifetime prevalence rate for schizophrenia ranges from 0.5 to 1.0% (APA, 1994). However, for schizophrenia the prevalence rate appear to be very similar in different countries and cultures (Stimmel, 1996).

Furthermore, epilepsy which is a major neurological disorder characterised by recurrent seizures has a lifetime prevalence of 5% (Sander and Shorvon, 1996; Raza *et al.,* 2001). Common causes include infectious, traumatic, metabolic or tumoral conditions or it may be idiopathic, that is unrelated to any underlying causes other than a possible hereditary predisposition (Engel, 2001). Falciparum malaria however, is a common cause of seizures in children living in malaria endemic areas (Ogutu and Newton, 2004). About 30% of children hospitalized with malaria have a history of convulsions (Wattanagoon *et al.*, 1944; Waruiru *et al.,* 1996). Also, in endemic areas, malaria parasitemia is found in 70% of children presenting with convulsions (Ogutu and Newton, 2004). While in cerebral malaria (CM) over 80% children are admitted with a history of convulsions (Molyneux *et a.l*, 1989) and over 60% have convulsion detected after hospitalization. Furthermore, seizures in malaria are associated with a poor outcome. Prolonged seizures in children with CM however, are

associated with neurological, cognitive and language deficits and the development of epilepsy (Holding *et al.,* 1999; Carter *et al.,* 2003). It can be said therefore that, sub-Saharan Africa (Nigeria inclusive) may have a higher prevalence of epilepsy being malaria endemic.

The mainstay in the management of patients with either neurological or neuropsychiatric disorders is in the use of centrally acting drugs. However, chemotherapy with centrally acting drugs is associated with the development of massive side effects. Moreover, the drugs are to be used over a long period of time. The adverse effect profile may be partly due to the fact that, the organisation and function of the CNS is very complex. Several neurotransmitters may be involved in the pathogenesis of these disorders. It is therefore difficult to find agents that will act selectively to produce a desired effect.

A classical example is the development of extrapyramidal symptoms (EPS) during antipsychotic treatment with typical antipsychotics such as chlorpromazine and haloperidol. The extrapyramidal symptoms characterised by dystonic reactions (oculogyric crisis, torticollis, opisthotonus, trismus), Pseudoparkinsonism (akinesia, tremor) and Akathisia (inability to sit still, continuous agitation, rocking and shifting of weight e.t.c.), are the most significant adverse effects in terms of frequency and reason for patient's non-compliance with antipsychotic drug therapy (Stimmel, 1996). EPS may be severe enough to require drug treatment itself. These effects are primarily due to blockade of dopamine D_2 receptors in areas of the brain other than the mesolimbic. Antagonism of D_2 receptors in the mesolimbic however, results in antipsychotic activity (Stimmel, 1996).

The newer or 'atypical' antipsychotics (clozapine, risperidone, Olanzapine etc) have shown good result in the treatment of refractory schizophrenia (Praharaj *et al.,* 2005). However,

their widespread use is limited also by side effects such as, a rare but severe agranulocytosis, seizures, hypotention and sialorrhoea (Foster and Olajide, 2005). Clozapine-induced sialorrhoea occurs in approximately 30% of pateients (Rogers and Shramko, 2000). Metabolic adverse effects, such as obesity, hyperlipidaemia, type 2 diabetes mellitus and an increased risk of insulin resistance also pose a serious burden (Koro *et al*.,2002; Kornegay *et al*.,2002; Sernyak *et al*.,2002 ; Best *et al.*, 2005; Rampes *et al*., 2005).

Similarly, patients with epilepsy fail to experience adequate control of their seizures despite the optimal use of available antiepileptic drugs -AEDs (Stables and Kupferberg, 1997). Synthetic AEDs are effective only in approximately 50% of patients and many refractory cases of epilepsy still remain highly resistant to their treatment (Heinemann *et al.*, 1994; Shorvon, 1996).Furthermore, AEDs are associated with side effects, including teratogenicity, chronic toxicity and adverse effects on cognition and behaviour (Smith and Bleck, 1991; Mattson, 1995; Devinsky, 1995; Samren *et al*., 1997; Raza *et al*., 2001).

Consequently, the argument outlined above and other reasons necessitate the need for the development of newer agents with greater efficacy, negligible or reduced side effects and devoid of unfavourable interactions to treat neuropsychriatric and neurological disorders.

1.3 THEORETICAL FRAMEWORK

Development of new drugs often starts with discovery and followed by screening. Screening of drugs involves scanning (observation of gross behaviour of animals) and evaluation. In simple screening, one or two tests are used to find substances with pharmacological activity. Evaluation, however involves determination of toxicity and potency.

The usual practice is to begin with toxicity studies. Investigation of the acute toxicity, is the first step in the toxicological investigation of an unknown substance. The index of the acute toxicity is the LD_{50}. The LD_{50} of *Randia nilotica* was determined using the method of Lorke (1983). This method, which can be used for every route of administration, is superior to others (Graphical calculation of Miller and Tainter - 1944; Arithmetic method of Karber – 1931 etc), because it uses fewer animals. With thirteen experimental animals, it is possible to obtain adequate information on the acute toxicity and on the LD_{50} (Lorke, 1983). Screening of the drug for psychotropic activity was also done during the LD_{50} determination (Irwin, 1968).

The result of the screen enabled the conduct of specific behavioural tests to evaluate the depressant effect of the extracts of *randia nilotica* (Turner, 1965).

i. Potentiation of pentobarbitone sleeping time (Turner, 1965b).

The test is used to elucidate CNS- active properties of drugs. Sedatives, hypnotics and tranquilisers as well as antidepressants are known to prolong pentobarbitone sleeping time (Vogel and Vogel, 1997). Neuroleptics (chlorpromazine, haloperidol) also prolong pentobarbitone sleeping time at low doses. However analeptics and stimulants (amphetamine) shorten sleeping time. A modified method, which uses Diazepam instead of pentobarbitone described by Rakotonirina *et al.*, (2001) was also used.

ii. Hole-board test.

This is employed to evaluate certain components of behaviour of mice, such as curiosity or exploration (Boissier *et al.,* 1964; Boissier and Simon, 1964). Poking the nose into a hole is a typical behaviour of the mice indicating a certain degree of curiosity. Benzodiazepines tend

to suppress nose poking at relatively low doses. The modified method described by Yemitan *et al.* (2001) was used.

iii. Rotarod method (Martin *et al.*, 1993)

This is used to evaluate the ability of drug to interfere with motor coordination. In 1957, Dunham and Miya suggested that, the skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod. Many central depressive drugs are active in this test. Anxiolytics and neuroleptics act at low doses. This test however does not differentiate between anxiolytics and neuroleptics but can only evaluate the muscle relaxant potency of the drugs.

Furthermore, the rotarod method has been used in toxicology to test for minimal neurotoxicity of a compound (Stables and Kupferberg, 1997). Untreated control mice, when placed on a 6 revolutions per minute rotating rod, can maintain their equilibrium for a prolonged period of time. Neurological impairment can be demonstrated by the inability of a mouse to maintain equilibrium for one minute in each of three successive trials.

iv. Mouse beam walking assay

This method offers improved sensitivity over the mouse rotarod in determining motor coordination deficits induced by psychotropic agents (Stanley *et al.*, 2005). It also has increased ability to predict clinically sedative doses of benzodiazepines evidenced by ataxia induced by these drugs which served as a predictor of sedative effects observed in the clinic.

v. Inhibition of amphetamine stereotypy in mice

Amphetamine is an indirect acting sympathomimetic agent which release catcholamines from its neuronal storage pools. In mice the drug induces a characteristic stereotypic behaviour characterised by continous sniffing, licking or gnawing, jumping and circling. Inhibition of amphetamine induced stereotypies in mice can be regarded as a simple method to detect neuroleptic acitivity (Vogel and Vogel, 1997).

vi. Pentylenetetrazol (Metrazol) antagonism in mice.

This assay has been used primarily to evaluate anti-epileptic drugs. However, it has been shown than most anxiolytic agents are also able to prevent or antagonise metrazol-induced convulsion (Lippa *et al.*,1979, Chapoutheir and Venault, 2001).Stimulants, antidepressants, neuroleptics and some antiepileptics do not show metrazol antagonism at tolerable doses (Lippa *et al*., 1979).

The anti-epileptic model primarily identifies compounds that raise seizure threshold and is a fairly good index of effectiveness against absence seizures –petit mal (Rang and Dale, 1995). With some minor exceptions, the pharmacological profile of the subcutaneous pentylenetetazol (ScPTZ) seizure model is consistent with the human condition (Snead 1992; Swinyard *et al.*, 1989). Like other rodent models of absence seizures, PTZ-induced seizures are potentiated by gammaminobutyric acid (GABA) agonist.

vii. Strychnine – induced convulsions in mice

The convulsant action of strychnine is due to interference with postsynaptic inhibition mediated by glycine. Glycine is an important inhibitory transmitter to motorneurones and interneurons in the spinal cord. Strychnine acts as a selective, competitive antagonist to

block the inhibitory effects of glycine at all glycine receptors. Strychnine sensitive post-synaptic inhibition at 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) and anti-epileptic effects (Larson, 1969).

viii. Electroshock convulsion test in mice and chicks (Swinyard, 1972; Swinyard *et al.,* 1952; Browning, 1992)

The electroshock assay is used primarily as an indication for compounds which are effective in *grand mal* epilepsy (Rang and Dale, 1995). Tonic hind limb extensions are evolved by electrical stimuli which are suppressed by antiepileptics but also by other centrally active drugs. The behavioural and electrographic seizures generated in this model are consistent with the human disorder (Swinyard *et al.,* 1989).

ix. Tail suspension test in mice

This test has been described by Steru *et al.* (1985) as a facile means of evaluating potential antidepressants. The immobility displayed by rodents when subjected to an unavoidable and inescapable stress has been hypothesized to reflect behavioural despair, which in turn may reflect depressive disorders in human.

Clinically effective antidepressants reduce the immobility that mice display after active and unsuccessful attempts to escape when suspended by the tail. It has been reported however, that several mouse strains are essentially resistant to tail suspension induced immobility (Vogel and Vogel, 1997).

x. Despair swim test in rats

The use of this to test for antidepressant activity was described by Porsolt *et al* (1977a, b, 1978). It was suggested that mice forced to swim in a restricted space from which they cannot escape are induced to a characteristic behaviour of immobility. This immobility reflects a state of despair which can be reduced by several agents that are therapeutically active in human depression. This model is suitable also for evaluating drugs against schizophrenia, since depression is a component of the disease – negative symptom (Noda *et al.*, 1995).

xi. Electroencephalographic (EEG) analysis from rat brain

Field potential anlaysis of rats by radioencephalography from different brain areas has been developed as a sensitive method in pharmacology for evaluating the effect of a compound on motility and or behaviour. (Dimfel *et al.*, 1986, 1990, 1992,; Kropf *et al.*, 1991).

xii. Phytochemical screening

The relationship between structure and activity has been shown by many authors. For example, CNS activity has been demonstrated in medicinal plants which contain saponins, flavonoids and tannins (Woo *et al*., 1980). It is also known that the composition and or concentration of these secondary metabolites depend on the environment in which they are grown.Thus the chemical composition of *Randia nilotica* will be re-evaluated. Phytochemical methods described by Trease and Evans (1989) will be used.

Separation and purification of crude extract will also be carried out using thin layer chromatographic (TLC) and column chromatographic techniques. The fractions will be screened for psychotropic activity.

xiii. Statistical analysis

Analysis of data generated will be conducted using the student's t-test and significant differences will be determined using one- way analysis of variance (ANOVA) to be followed by Dunnet's and Scheffe's post-hoc tests. All results will be expressed as means ± standard error of the mean (±SEM). Values that are $\leq$ 0.05, 0.01 or 0.001 will be considered significant.

1.4 OBJECTIVES OF THE STUDY

The main objective of this research is to establish scientific bases for the uses of *randia nilotica* in traditional medicine for the treatment of neurodegerative and neuropsychiatric illnesses.

Other specific objectives include:

- Determine relative acute toxicity profile of *Randia nilotica* extracts and its fractions in rats and mice;
- Establish the precise mechanism of CNS activities of extracts of *Randia nilotica* in rodents;
- Determine which part of *randia nilotica* is more potent in its CNS effects;
- Determine active chemical substances present in the stambark of *randia nilotica*;
- Determine the minimal neurotoxicity level of *randia nilotica* and some of its metabolites;
- Determine whether *Randia nilotica* is worthy of further attention in the development of a new compound to be used in treatment of neuropsychiatric and neurological diseases.

1.5 STATEMENT OF RESEARCH HYPOTHESIS

Randia nilotica stapf. contains pharmacologically active substances which are useful in the treatment of neurodegenerative and neuropsychiatric diseases.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Drugs that act on the central Nervous system (CNS)

Drugs that act on the CNS were among the first to be discovered by primitive humans and are still the most widely used group of pharmacologic agents (Nicoll, 2004). In addition to their use in therapy, many drugs acting on the CNS are used without prescription to increase one's sense of well-being.

The mechanism by which various drugs act in the CNS have not always been clearly understood since the causes of many of the conditions for which these drugs are used (schizophrenia, anxiety e.t.c.) are themselves poorly understood. Recent advances on CNS Pharmacology have now shown clearly that, nearly all drugs with CNS effects act on specific receptors that modulate synaptic transmission (Nicoll, 2004).

Studies of drugs acting on CNS are important for several reasons. Firstly, drugs are among the most important tools for studying all aspects of CNS physiology, from the mechanism of convulsions to the laying down of long-term memory. Secondly, unravelling the actions of drugs with known clinical efficacy has led to some of the most fruitful hypotheses regarding the mechanism of disease. For example, information on the action of antipsychotic drugs on dopamine receptors has provided the basis for important hypotheses regarding the pathoyphysiology of schizophrenia. Similarly, studies on the effects of a variety of agonists and antagonists on gammaminobutyric acid (GABA) receptors are resulting in new concepts pertaining to the pathophysiology of several diseases, including anxiety and epilepsy. Plants were and remain an important source of drugs acting on CNS.

2.2 Anxiolytic and Hypnotic drugs

Anxiety is an unpleasant state of tension, apprehension or uneasiness – a fear that seems to arise from an unknown source. Disorders involving anxiety are the most common mental disturbances. The symptoms of severe anxiety are similar to those of fear (such as tachycardia, sweating, trembling, and palpitations) and involve sympathetic activation (Howland and Mycek, 2006).

The symptoms of severe, chronic, debilitating anxiety may be treated with antianxiety drugs (sometimes called anxiolytics or minor tranquilizers). Becaue all of the antianxiety drugs also cause some sedation, the same drugs often function clinically as both anxiolytic and hypnotic (sleep-inducing) agents. In addition, some have anticonvulsant action (van Boxtel and Buitenhuis, 2001).

Commonly used antianxiety medications include the following classes: benzodiazepines (BZD), azaspirodecanedione derivatives, beta blockers, tricyclic antidepressants (TCAs), monoamine oxidase inhibitors (MAOIs), selective serotonin reuptake inhibitors – SSRIs (Cates *et al.*, 1996).

2.2.1 Benzodiazepines

These are the most widely used anxiolytics. Currently available BZDs include: alprazolam, chlordiazepoxide, clonazepam, clorazepate, diazepam, flurazepam, oxazepam, temazepam, triazolam.

The target for the benzodiazepine action is the $GABA_A$ receptors (GABA is the major inhibitory neurotransmitter in the CNS). These receptors are composed of α, β and γ subunit

families of which a combination of five or more span the postsynaptic membrane. BZDs modulate GABA effects by binding to a specific, high affinity site located at the interface of the α-subunit and the γ_2 subunit. These receptors are sometimes designated as BZ_1 or BZ_2 depending on their affinity for a particular α-subunit (Howland and Mycek, 2006).

The BZD receptor locations on the CNS parallel those of the GABA neurones. Binding of GABA to its receptor triggers an opening of a chloride channel which leads to an increase in chloride conductance. BZDs increase the frequency of channel openings produced by GABA. The influx of chloride ions causes a small hyperpolarisation that moves the postsynaptic potential away from its firing threshold and thus, inhibits the formation of action potentials. BZDs and GABA mutually increase the affinity of their binding sites without actually changing the total number of sites. The clinical effects of the various BZDs correlate well with each drug‘s binding affinity for the GABA receptor-chloride ion channel complex.

The most important indications for BZDs as a whole are anxiety states and insomnia. For the short acting agents (oxazepam, lormetazepam, triazolam, temazepam) indications also include sedation and even light anesthesia in peri-operative states. Longer acting agents (chlordiazepoxide, clorazepate, flurazepam e.t.c) are used for the management of alcohol withdrawal. Some are specifically used in epilepsy (clonazepam, diazepam), sometimes in combination with other anticonvulsant therapy or alone for the discontinuation *of status epilepticus.* Diazepam is especially useful for the relief of muscle spasm in various disorders. The effects on sleep are a decrease in sleep latency, a diminished number of awakenings with, as an overall effect, an increase in total sleep time.

The most common adverse effects are drowsiness, ataxia and reduced psychomotor performance. Moreover, adverse effects also include dependence and thus drug abuse. Tolerance develops within 3 months for anxiety. Long term use can result in withdrawal symptoms such as insomnia, anxiety, tinnitus, tremor, perceptual disturbance and loss of appetite (van Boxtel and Buitenhuis, 2001).

The hypnotic zolpidem is not a BZD, but acts on a subset of a BZD receptor family BZ_1. Zolpidem has no anticonvulsant or muscle relaxing properties. It shows no withdrawal effects, exhibits minimal rebound insomnia, and little or no tolerance occurs with prolonged use. Adverse effects (AEs) are nightmares, agitation, headache, gastrointestinal (GI) upset, dizziness and day time drowsiness. Zaleplon is very similar to zolpidem in its hypnotic actions, but it causes fewer residual effects on psychological and cognitive functions compared to zolpidem or BZDs.

2.2.2 Azaspirodecanedione derivatives

Buspirone is the first representative of this group. Buspirone acts as a partial agonist at the postsynaptic 5-Hydroxytryptamine (5-HT_{1A} receptor and a full agonist at presynaptic 5-HT_{1A} receptors (Yocca, 1990). This results in decreased firing of 5-HT neurones. It is licensed for the treatment of generalized anxiety (Wilson *et al.*, 2005). It does not affect the GABA neurotransmitter system, but has efficacy comparable to that of the BZDs (Rickels, 1990). Buspirone displays some affinity for dopamine (DA_2) and 5-HT_{2A} receptor.

Buspirone lacks anticonvulsant and muscle relaxant properties of BZDs and causes only minimal sedation. At least 1 -2 weeks are required before onset of antianxiety activity and maximal effects may require up to six weeks. It undergoes metabolism by CYP3A4

isoenzymes in the liver. Common adverse effects are dizziness, nausea and headache occurring with a frequency greater than 5%. Nervousness and light-headedness were also reported. Psychomotor and cognitive dysfunctions are minimal and dependence is unlikely.

Eptapirone is a new 5-HT_{1A} recptor agonist with a greater intrinsic activity than Buspirone (Wilson *et al.,* 2005). Preclinical *in vitro* studies have shown that eptapirone has an affinity for $5HT_{1A}$ binding sites higher than buspirone and somewhat lower than that of the reportedly full $5HT_{1A}$ agonist, flesinoxan. More importantly, it is more selective for $5HT_{1A}$ sites than either buspirone or flesinoxan.

2.2.3 Adrenergic Beta-receptor blockers

Propronolol, which is a non specific beta-adrenoceptor blocker, is considered to be a less effective antianxiety agent than BZDs. Propranolol may be considered useful in patients with prominent cardiovascular symptoms of anxiety, such as tachycardia, palpitations and tremor (Dubovsky, 1990).

Single dose beta-blocker therapy is likely to be effective for performance anxiety (Agras, 1990). Atenolol appeared to be more helpful in discrete social phobia (SP) than in generalized SP in a placebo controlled study. Hwoever, the sample size of the subtype was too small to draw definitive conclusions (Liebowitz *et al.*, 1992).

Propranolol treated post-traumatic stress disorder (PTSD) patients were reported to have fewer intrusive thoughts and nightmares and to experience less explosiveness and autonomic instability (Kolb *et al.*, 1984).

2.2.4 Tricyclic antidepressants (TCAs)

TCAs are not traditionally considered to be treatments for anxiety disorders, however a study revealed the comparable efficacy of imipramine, trazodone and diazepam in treatment of generalized anxiety disorder (GAD) patients who did not have a comorbid major depression or panic disorder (Rickels *et al.,* 1993).

Imipramine is the most widely studied TCA in treating PD. Clomipramine appears to be more superior in this regard (Brawman – Mintzer and Lydiard, 1994). Clomipramine is also effective in treatment of obsessive compulsive disorder. Imipramine and amitriptyline have the most solidly established efficacy in treatment of core-intrusive post – traumatic stress disorder (PTSD) symptoms (Sutherland and Davidson, 1994).

Tricyclics block the amine (norepinephrine or serotonin) reuptake pumps, which terminate amine neurotransmission. Such an action presumably permits a longer sojourn of neurotransmitter at the receptor site. The most common adverse effects seen with TCAs include sedation, orthostatic hypotension and anticholinergic effects. A hyperstimulatory reaction characterised by intensification of anxiety symptoms, agitation, tachycardia and insomnia has been reported in about 20% of patient taking TCAs (Noyes *et al.,* 1989)

Concurrent use of monoamine oxidase inhibitors (MAOIs) with TACs has resulted in hypertensive crisis, hyperpyretic episodes, convulsions and death. Concurrent use of sympathomimetis with TCAs may potentiate cardiovascular effects, possibly resulting in arrythmias, tachycardia or severe hypertension. Medications that can increase plasma TCA levels, possibly resulting in toxicity, include cimetidine, phenothiazines, fluoxetine, sertraline and paroxetine (USP-DI, 1994).

Mirtazapine is a novel antidepressant in a new class referred to as the noradrenergic and specific serotonergic antidepressants (NaSSA). It influences both central noradrenergic and serotonergic neurotransmission by directly inhibiting noradrenergic α2-autoreceptors and α2-heteroreceptors (deBoer, 1995). Mirtazapine also selectively inhibits specific postsynaptic 5-HT_2 and 5-HT_3 receptors and histamine- H_1 receptors, and this contributes to its favourable tolerability profile (Fava *et al.,* 2001). A recent study had shown the effectiveness of mirtazapine in the treatment of GAD (Goodnick *et al,* 1999; Gambi *et al.,* 2005). Its efficacy in PTSD has been established (Davidson *et al.,* 2003; Chung *et al.,* 2004) as well as in PD (Ribiero *et al.,* 2001; Sarchiapane *et al.,* 2003).

The side effect profile of mirtazapine was very favourable. The most frequently observed side effects are increase in appetite, weight gain and drowsiness (Connor *et al.,* 1999; Thase *et al.,* 2001; Roose *et al.,* 2003; Gambi *et al.,* 2005). Trazodone and Nefazodone are believed to have a similar mechanism of action with mirtazapine.

2.2.5 Monoamine oxidase inhibitors (MAOIs).

Typical MAOIs are phenelzine, tranylcypromine, isocarboxazid and meclobemide. MAOIs block the oxidative deamination of monoamines, that is, norepinephrine and serotonin by inhibiting monoamine oxidase type A (MAOA) and dopamine also by monoamine oxidase type B (MAOB) inhibition, thereby increasing these neurotransmitters at their receptors in the brain and in the periphery. MAOA is also located in the gut and liver and it metabolises tyramine from the diet.

Phenelzine blocked panic attacks in 100% of PD patients and 95% of agoraphobic patients in an open trial (Buigues and Vallejo, 1987). Unfortunately, the side effect profile and

dietary restrictions preclude the routine use of MAOIs in most PD patients despite good efficacy. Generalized SP patients were also shown to be responsive to phenelzine (Liebowitz *et al.,* 1992). Several open studies suggested possible effectiveness of phenelzine in treating PTSD. Treatment- responsive symptoms included nightmares, flashbacks and intrusive recollections but not avoidance (Frank *et al.,* 1988).

Common adverse effects of MAOIs include weight gain, orthostatic hypotension, edema and sexual dysfunction (acronym: WOES). MAOIs cause fewer anticholinergic effects than TCAs but more problematic orthostatic hypotension (Rabkin, 1984). Because of this side effect profile as well as dietary and drug restrictions, patients generally prefer other antianxiety medications to MAOI therapy (Cates *et al.,* 1996).

The serotonin syndrome and hypertensive crisis are potentially lethal reactions attributed to drug interactions with MAOIs. The serotonin syndrome is a hyperserotonergic state manifested by confusion, restlessness, myoclonus, hyperreflexia, diaphoresis, shivering, tremor, diarrhoea and/or fever. Drugs such as clomipramine (and other TCAs), meperidine, SSRIs, tryptophan, dextromethorphan, and trazodone can cause the serotonin syndrome when taken concurrently with MAOIs (Cates *et al.,* 1996).

Hypertensive crisis manifested by hypertension, severe headache, stiff or sore neck, nausea or vomiting can be caused by using MAOIs with sympathomimetics (ephedrine, pseudoephedrine, amphetamines, phenylephrine, phenylpropanolamine, levodopa etc), tyramine containing foods and TCAs (Maxmen, 1991).

2.2.6 Selective serotonin reuptake inhibitors (SSRIs)

Currently available SSRIs include fluoxetine, sertraline, paroxetine, fluvoxamine, zimelidine, venlafaxine and citalopram.

Controlled studies have revealed positive effects of fluoxetine, sertraline and fluvoxamine on obsessive-compulsive disorder (OCD). Fluoxetine was found to be as effective as clomipramine in treating OCD, but with fewer side effects (Pigott *et al.,* 1990). SSRIs have also been used successfully in the treatment of PD. In one study, patients show moderate-to marked improvement with fluoxetine (Schneir *et al.,* 1990).

A significant reduction in symptoms occurred when PTSD patients took fluoxetine in a double-blind, placebo controlled study. Of particular interest is the apparent ability of the SSRIs to treat the avoidance and numbing symptoms of PTSD in trials thus far (Sutherland and Davidson, 1994). Large placebo-controlled trials revealed efficacy for sertraline (Brady *et al.,* 2000) and paroxetine (Tucker *et al.,* 2001) in PTSD, but not all patients respond optimally to SSRI treatment. Three open trials have examined fluoxetines efficacy in social phobia (SP), and all yielded moderately effective results (Liebowitz, 1993).

SSRIs are selective blockers of the re-uptake of serotonin at presynaptic neurones and have little if any effects on muscarinic, histaminergic, adrenergic or serotonergic receptors. They are as effective as TCAs with fewer cardiovascular effects. SSRIs are relatively free of anticholinergic, orthostatic and sedative effects. Sexual dysfunction is a common side effect of SSRIs. Common reasons for the discontinuation of SSRIs include nervousness, nausea, insomnia and anxiety (Guze *et al.,* 1992).

2.2.7 Miscellanious sedative-anxiolyic drugs

Barbiturates have been used as mild sedatives to relieve anxiety, nervous tension and insomnia. When used as hypnotics, they suppress rapid eye movement (REM) sleep more than other stages. However, most have been replaced by the BZDs.

Several histamine H_1 antagonists, e.g. hydroxyzine, promethazine and mepyramine display considerable sedative effects and they are sometimes used as sedative-hypnotics. Meprobamate is also a sedative hypnotic drug. However it has less anxiolytic effect than the BZDs and it may cause serious CNS depression.

2.3 ANTIPSYCHOTICS (Neuroleptic drugs).

Neuroleptic drugs (also called antischizophrenics, antipsychotic drugs or major tranquilisers) are used primarily to treat schizophrenia, but they are also effective in other psychotic states, such as manic states and delirium.

Schizophrenia is a particular type of psychosis i.e., a mental disorder caused by some inherent dysfunction of the brain. It is characterised by delusions, hallucinations (often in form of voices) and thinking or speech disturbances. The illness often initially affects people during adolescence and is a chronic and disabling disorder. Schizophrenia has a strong genetic component and probably reflects some fundamental biochemical abnormality, possibly an overactivity of the mesolimbic dopaminergic neurones.

The neuroleptic drugs represent several diverse, heterocyclic structures with markedly different potencies. The antipsychotics are classified into phenothiazine, thioxanthene, butyrophenone, dibenzoxazepine, dihydroindolone, dibenzazepine and benzisoxazole groups.

The traditional antipsychotic drugs (Chlorpromazine, haloperidol etc) referred to as typical are generally very effective in treating positive symptoms (hallucination, delirium, bizarre behaviour etc) of schizophrenia, but are less effective in treating negative symptoms (alogia, anhedonia, avolition, affective blunting etc). The newer or atypical antipsychotic drugs (clozapine, risperidone etc) are effective in treating positive symptoms but are more effective in treating negative symptoms.

2.3.1 Phenothiazines

These are further subdivided into aliphatic, piperidine and piperazine derivatives.

2.3.1.1 Aliphatic phenothiazines

Chlorpromazine (CPZ) is the best known representative of the aliphatic phenothiazines. It is one of the most sedative antipsychotic agents and is therefore very effective in the treatment of agitated and violent patients (van Boxtel and Buitenhuis, 2001).

Pharmacological investigation showed that CPZ blocked the actions of many different mediators including histamine, catecholamines, acetylcholine (ACh.) and 5-hydroxytryptamine (5-HT), and this multiplicity of action led to the trade name 'largactil'. It is now clear that antagonism at dopamine receptor is the main determinant of antipsychotic action (Rang and Dale, 1995).

Extrapyramidal effects are seen with a rather low incidence. However, CPZ displays marked anticholinergic activity. There have been reports of hepatotoxicity, also in patients with previously normal hepatic function. Alimemazine and trifluopromazine are other representatives from this group.

2.3.1.2 Piperidine Phenothiazines

The piperidines (thioridazine, pipothiazine and pericyazine) have the lowest potential to cause extrapyramidal effects.

Thioridazine is one of the most sedative phenothiazines. It may decrease the inotropic activity of digitalis by its quinidine – like action, which can cause myocardial depression. With thioridazine drug induced sexual dysfunction and especially cardiotoxicity with prolongation of the QT – interval are more frequently seen than with other phenothiazines. Anticholinergic properties are also high (van Boxtel and Buitenhuis, 2001).

2.3.1.3 Piperazines

The piperazines include fluphenazine, trifluoperazine, prochlorperazine, perazine and perphenazine.

These are agents with a high antipsychotic potency with less pronounced anticholinergic effects. However, their potential to produce extrapyramidal effects is more pronounced.

Fluphenazine is a short-acting agent. For the management of agitated and potentially violent patients, its hydrochloride formulation is frequently used for parenteral administration. Although its principal pharmacological activities are similar to those of the other phenothiazines, fluphenazine displays only weak sedative action and it shows little anticholinergic and hypotensive effects.

Trifluoperazine is also a more potent antipsychotic than CPZ with only minor sedative, anticholinergic and cardiovascular activity.

2.3.2 Thioxanthene derivatives

The pharmacological profiles of flupenthixol and zuclopenthixol are similar to those of the piperazine-type phenothiazines.

Chlorprothixene has affinity for both dopamine –D_2 receptors and 5-HT_2 receptors but also for α-adrenergic and histamine H_1 receptors. It is used for the treatment of psychosis but also for sedation and for the management of manic states (van Boxtel and Buitenhuis, 2001). Thiothixene is used for the same indications but is less sedative than chlorprothixene.

2.3.3 Butyrophenones

Haloperidol, benperidol, droperidol, bromperidol and pipamperon are representatives from this group.Except for pipamperon, they have high affinity for the postsynaptic D_2 receptors. Haloperidol has similar pharmacological properties as the piperazine phenothiazines. It also has the same indications as the phenothiazines. Haloperidol is considered the agent of choice for the management of acute psychotic states with agitation and possible violence.

Haloperidol has very little anticholinergic effects and it is also less sedating than CPZ, for example. It does not result in type 2 diabetes mellitus as does the atypical antipsychotics like clozapine (Best *et al.*, 2005).

Benperidol is used mainly to diminish libido in the management of antisocial behaviour. Droperidol is one of those antipsychotics that is used in anaesthetic practice to potentiate

analgesic and anaesthetic effects of other agents. Pimozide, penfluridol and fluspirilene are diphenylbutylpiperidine derivatives.

2.3.4 Dibenzoxazepines

These include such drugs as chlothiapine, metiapine, loxapine, and zotapine. They are typical antipsychotics with high antidopaminergic activity (van Boxtel and Buitenhuis, 2001). Their pharmacological actions do not differ from those of the other neuroleptics and they are used for the same indications.

Chlothiapine is notorious for causing extrapyramidal reactions. Its parenteral use is only idicated for agitated schizophrenic patients and organic psychosis and for some patients with acute mania.

2.3.5 Dibenzazepines

Clozapine is an 'atypical' antipsychotic which is used for the treatment of schizophrenia. It is primarily indicated for schizophrenic patients with predominantly negative symptoms. Its indication can be extended to conventional neuroleptics (Foster and Olajide, 2005). It can also be substituted for other antipsychotics in patients with serious extrapyramidal symptom as it has little tendency to provoke these (Rampes *et al.,* 2005).

Clozapine is a highly sedating agent. However, it has a dose related risk of inducing seizures in non-epileptic patients (Pies, 1988; Davinsky *et al.,* 1991; Berman *et al.,* 1992; Wilson and Claussen 1994; Toth and frankenburger, 1994). Haematological monitoring of white blood cell count is mandatory and is also a condition for clozapine being dispensed becaue the drug carries a 0.73% risk of neutropenia (Munro *et al.,* 1999). Other unwanted effects are rare but

severe and include agranulocytosis, orthostatic hypotension and sialorrhoea. Clozapine-induced sialorrhoea occurs in about 30% of patients (Rogers and Shramko, 2000). Metabolic adverse effects, such as obesity, hyperlipidaemia and diabetes mellitus also pose a serious threat with clozapine therapy (Koro *et al.,* 2002; Leonard *et al.,* 2002).

Clozapine has more affinity for D_1 and D_4 receptor blockade than D_2 receptor blockade and also has serotonergic$_2$ (5-HT_2) receptor blockade. It has been suggested that the combinationof D_2 and 5-HT_2 blockade and/or the D_1 and D_4 effects accounts for the drug's unique effects (Lieberman, 1993). Fluperlapine and Olanzapine have a lesser potency and have a relatively low affinity at most dopamine receptors, but they do bind to muscarinic, 5-HT_2, α-adrenergic and H_1- histamine recptors.

2.3.6 Benzisoxazole derivatives

The heterocyclic antipsychotic agent risperidone, is a benzisoxazole derivative with potent antiserotonergic (5-HT_2) receptor blockade as well as weak antidopaminergic (D_2) receptor antagonism (Janssen *et al.*, 1988; Chouinard and Arnott, 1993). These characteristic features of risperidone are probably responsible for fewer extrapyramidal side-effects than with the use of typical antipsychotics like haloperidol (Wirshing *et al.*, 1999; Zhang *et al.,* 2001).

The most common adverse effects with risperidone therapy include agitation, anxiety, isomnia, EPS, headache and nausea. Orthostatic hypotension is possible during initial dose titration (Cohen, 1994, Marder and Meibach, 1994).

A study has shown clear evidence of higher risperidone total plasma concentrations for patients over 40 years of age. This linear increase (over30% per decade) may then lead to an

increased incidence of adverse effects in elderly patients. (Aicchorn *et al.*, 2005). However, there was no significant sex-related differences in the average plasma concentration of risperidone as observed in this study.

Risperidon appears to be safe treatment option for breastfeeding mothers and their infants. In a case study, a patient responded well to antipsychotic therapy with risperidone. Her infant did not display any adverse effects, and psychomotor devleopment was normal (Aicchorn *et al.*, 2005b).

2.3.7 Miscellanious antipsychotics

Quetiapine is an antipsychotic agent with a structure related to that of the BZDs. It has high affinity for H_1 histamine receptors and intermediate affinity for 5 – HT_2 and dopamine D_2 receptors. Molindone is low potency typical antipsychotic with virtually no effect on α_1 receptor. It belongs to the dihydroindolone group.

Aripiprazole is a new atypical antipsychotic which is clinically effective and has a favourable side effect profile (Hirose *et al.*, 2004). Aripiprazole is a partial agonist at D_2 receptors (Burris *et al.*, 2002; Burris 2000; Lawler *et al.*, 1999). It's mechanism of action also includes 5-HT_{2A} recptor antagonism (Kikuchi *et al.*, 1995) and partial agonism at 5-HT_{1A} receptors (Jordan *et al.*, 2002).

Unlike risperidone, olanzapine or typical antipsychotics, the efficacy of aripiprazole is not generally associated with EPS, sedation, weight gain, elevations in serum prolactin or cardiovascular side effects. These favourable antipsychotic properties and a different mechanism of action distinguish aripiprazole from other atypical agents.

2.4 ANTIDEPRESSANTS

Major depression is one of the most common psychiatric disorders. The World Health Organisation (WHO) ranks unipolar depression as the fourth most important cause of mortality and disability (Murray and Lopez, 1997). Depression has an overall prevalence of 5.8% and the prevalence of late life depression is estimated to be 15% (Coppen and Bolander - Gouaille, 2005). Persons with neurodegenerative disorders are at high risk of depression. In Alzheimers disease, the frequency of depression is around 50% (Gottfries, 2001). The risk of suicide is calculated to be 20 times that of a general population, and between 50% and 70% of all people who commit suicide suffer from depressive illness (Barraclough *et al.*, 1974; Isaccson, 2000).

The symptoms of depression are often subtle and unrecognized both by patients and by physicians. Patients with vague complaints that resist explanation as manifestations of somatic disorders and those who might be simplistically described as "neurotic" should be suspected of being depressed.

Depression is a heterogenous disorder that has been characterised and classified in a variety of ways. According to the American Psychiatric Associations fourth edition (1994) of the *Diagnostic and Statistical Manual of Mental Disorders (DSM-IV),* several diagnoses of affective disorders are possible. Major depression and dysthymia (minor) are pure depressive syndromes, whereas bipolar disorder and cyclothymic disorder signify depression in association with mania. A simplified classification based on presumed origin is as follows:

i. "reactive" or "secondary" depression (most common occurring in response to real stimuli such as grief, illness etc.

ii. "Endogenous" depression, a genetically determined biochemical disorder manifested by inability to experience ordinary pleasure or to cope with ordinary life events; and

iii. Depression associated with bipolar affective (manic-depressive) disorder.

An intensive effort to formalize guidelines for the treatment of depression is provided by the cross-disciplinary publication of the Depression Guideline Panel (1993). Pharmacologic treatment is emphasized, though a continuing role for electro-convulsive therapy for delusional or severe forms of life-threatening depression is noted.

Although many new antidepressant drugs have been introduced recently, response rates have not improved and the treatment of depressive illness remain unsatisfactory (Coppen and Bolander-Gouaille, 2005). The selective serotonin reuptake inhibitors, as well as the older tricyclic antidepressants, have a response rate of approximately 50%, compared to 32% on placebo (Agency for Health Care Policy and Research, 1999; Gottfries, 2001). Furthermore, depressive illness is a periodic illness and length of the period of each episode can vary widely. The illness is also recurrent such that the life time morbidity can be considerable (Angst *et al.,* 2003). Despite intensive research, the mechanisms of action of various pharmacologic treatments are still not understood, though most are believed to involve effects on two monoamine neurotransmitters- serotonin and norepinephrine (Potter and Hollister, 2004).

The classes of drugs available in treatment of depression include the following: selective serotonin reuptake inhibitors, serotonin/norepinephrine re-uptake inhibitors, tricyclic

antidepressants, tetracyclic monoamine oxidase inhibitors, dibenzoxazepines, phenylpiperazines, monocyclic aminoketones and the use of lithium salts.

2.4.1 Selective Serotonin Re-uptake Inhibitors (SSRIs)

Typical SSRIs used in derpression are fluoxetine, sertraline, paroxetine, fluvoxamine, citalopram, escitalopram. They have virtually replaced TCA_S as first line agents in the treatment of depression. Although no more effective than TCA_S, patient tolerance of adverse effects is better with SSRIs and they (SSRIs) are more convenient to use since the initial starting dose is often the effective dose, thus requiring no dosage titration (Montgomery *et al*., 1994).

SSRIs are potent and specific serotonin reuptake inhibitors with negligible binding to alpha or beta adrenergic, muscarinic, serotonin, dopamine or histamine receptors. Inhibition of reuptake of serotonin leads to increased concentrations of the neurotransmitter in the synaptic clefts and ultimately, to greater postsynaptic neuronal activity.

Antidepressants, including SSRIs, typically take two weeks to produce improvement in mood and maximum benefit may require twelve weeks or more (Howland & Mycek 2006).

Fluoxetine and paraxetine are potent inhibitors of a heptatic cytochrome P450 isoenzyme (CYP 2D6) responsible for elimination of tricyclic antidepressant drugs, neuroleptic drugs, and some antiarrythmic and β-adrenergic antagonist drugs (Preskorn, 1993).

SSRIs have fewer and less severe adverse effects however, they can cause GI effects, weakness, sexual dysfunction, sleep disturbances and drug interactions. Paroxetine and

fluvoxamine are sedating, and may be useful in patients who have difficulty sleeping. Conversely, patients who are fatigued may benefit from one of the more activating antidepressants, such as fluoxetine.

Antidepressants should be used cautiously in children and teenagers, becaue about one out of 50 children become more suicidal as a result of SSRI treatment. Large intakes of SSRIs do not cause cardiac arrythmias, but fluoxetine may cause seizures. All SSRIs have the potential to cause a serotonin syndrome characterised by hyperthermia, muscle rigidity, myoclonus and changes in mental status and vital signs when used in the presence of MAOI.

2.4.2 Tricyclic Antidepressants

The tricyclic antidepressants (TCA_S) block norepinephrine and serotonin uptake into the neuron. The TCAs include the tertiary amines-imipramine, amitriptyline, clomipramine, doxepin and trimipramine. The TCAs also include the secondary amines-desipramine and nortriptyline. All have similar therapeutic efficacy, and the choice of drug depends on patient tolerance of side effects and duration of action. Patients who do not respond to one TCA may benefit from a different drug in this group. These drugs are a valuable alternative for patients who do not respond to SSRIs.

TCAs are potent inhibitors of the neuronal re-uptake of norpinephrine and serotonin into presynaptic nerve terminals. However at therapeutic concentrations, they do not block dopamine transporters. By blocking the major route of neurotransmitter removal, the TCAs lead to increased concentrations of monoamines in the synaptic cleft, ultimately resulting in antidepressant effects (Howland & Mycek 2006).

TCAs also block serotonergic, α-adrenergic, histaminic, and muscarinic receptors. As histamine H_1 receptor blockers doxepin, trimipramine and amitriptyline are several hundred times more potent than diphenhydramine. TCAs block muscarinic receptors, which leads to peripheral and central anticholinergic effects. Amitriptyline and nortryptyline are the most potent. α_1, adrenergic blockade helps explain the occurrence of postural hypotension and reflex tachycardia seen. Doxepine, amitriptylene are the most potent whereas protiptyline and desipramine are the least potent α_1 - adrenergic blockers.

Blockade of 5-HT re-uptake also contributes to orthostatic hypotension because of effects on serotonergic neurons in the brainstem vasomotor centre. Imipramine and nortriptyline have about equal activity at $\alpha_{1,}$ - adrenergic receptors, yet imipramine cause significantly more orthostatic hypotentsion than nortriptyline. The difference between these two drugs is that imipramine is a much more potent 5-HT reuptake blocker, and nortriptyline is more potent as a 5-HT receptor blocker. TCAs are very weak antagonists of dopamine (D_2) receptors, whereas beta-adrenergic, GABA, opiate and BZD receptors are not antagonised by TCAs (Richelson, 1982).

Chronic administration of antidepressants causes different effects on neurotransmitters and receptors than is seen with acute administration. The major functional consequence of chronic TCA treatment is a gradual facilitation of postsynaptic α_1- adrenergic receptor function, as well as increased physiologic sensitivity to 5-HT in the brain. There is also a down regulation (fewer receptor sites) and decreased sensitivity of α_2 – adrenergic autoreceptors and beta-adrenergic receptor function (Baldessarini, 1989).

2.4.3 Monoamine Oxidase Inhibitors (MAOIs)

MAOIs block oxidative deamination of monoamines i.e. norepinephrine and serotonin by inhibiting monoamine oxidase type A (MAOA) and dopamine also by monoamine oxidase type B (MAOB) inhibition, thereby increasing these neurotransmitters at their receptors in the brain and in the periphery (van Boxtel and Buitenhuis, 2000). MAOA is also located in the gut and liver and it metabolizes tyramine from diet.

Two MAOIs are currently available for treatment of depression: phenelzine and tranylcypromine. They are indicated for atypical depression and are an alternative treatment for patients with major depression who fail to respond to other antidepressants (Nies, 1984, Brown & Bryant, 1988). Changes in the neurotransmitter levels are seen in several days, but the clinical effect may lag by several weeks. .

Phenelzine is a non-selective hydrazine-type MAOIs while the also non-selective inhibitor tranylcypromine is of the non-hydrazine type phenelzine, tranylcypromine and isocarboxazid are irreversible inhibitors. Phenelzine is partly metabolised by acetylation and slow acetylators are more prone to toxicity. Moclobemide increases concentrations of serotonin and noradrenaline by means of reversible inhibition of MAOA.

MAOIs have a low therapeutic index. Adverse effects include orthostatic hypotension, impotence and insomnia. Overdoses become manifest by symptoms of agitation and hyperreflexia followed by convulsions. Rare, but serious cases of hepatotoxicity have been associated with the use of isocarboxazide and of phenelzine.

Interactions can be expected in all situations with risks for potentiation of sympathomi metic amine activity, particularly by indirect acting amines such as tyramine. Patients who are treated with MAOI must avoid food rich in tyramine (e.g. aged cheese, red wine, beer and vegetables such as beans). MAOIs block the metabolism of tyramine resulting in adrenergic over stimulation which may result in a hypertensive crisis (Shulman *et al.,* 1989; Brown and Bryant, 1988).

Interactions with foods containing tyramine is reduced with selective MAOA inhibitors such as moclobemide. MAOI can potentiate or prolong the action of TCAS but also of CNS depressants such as barbiturates. Accumulation of the epileptogenic metabolite of the opiod meperidine has been described (Van Boxtel and Buitenhuis, 2000). Other drugs which can cause hypertensive crisis when combined with MAOIs include sympathomimetics (Ephedrine, phenylpropanolamine); stimulants (amphetamine, methylphenidate); levodopa, buspirone and venlafaxine and SSRIs (Walker *et al*., 1984).

2.4.4 Serotonin/Norepinephrine Re-uptake Inhibitors

Venlafaxine and duloxetine selectively inhibit the re-uptake of both serotonin and norepinephrine (NE). These agents, termed selective serotonin/norepinephrine reuptake inhibitors (SNRIs), may be effective in treating depression in patients in which SSRIs are ineffective.

Furthermore, depression is often accompanied by chronic painful symptoms (neuropathic pain), such as backache and muscle aches, against which SRRIs are also relatively ineffective. The pain is, in part, modulated by 5-HT and NE pathways in the CNS. SNRIs and TCAs with their dual actions of inhibiting both 5-HT and NE reuptake, are often

effective in relieving physical symptoms of neuropathic pain. SNRIs unlike the TCAs, have no activity at adrenergic, muscarinic, or histamine receptors and, thus have fewer adverse effects than the TCAs (Howland and Mycek, 2006).

Venlafaxine is a potent selective inhibitor of 5-HT reuptake and at higher doses is a selective inhibitor of NE receptor (Beique *et al.*, 1999; Bymaster *et al.*, 2001). It is also a mild inhibitor of dopamine reuptake. Venlafaxine has minimal inhibition of the cytochrome P450 isoenzymes. The most common side effects of venlafaxine are nausea, dizziness, insomnia, sedation and constipation. At high doses, there may be an increase in blood pressure.

Duloxetine inhibits 5-HT and NE re-uptake at all doses. It should not be administered to patients with hepatic insufficiency. Metabolittes are excreted in the urine, and the use of duloxetine is not recommended in patients with end-stage renal disease. Food delays the absorption of the drug. G1 side effects are common with duloxetine including nausea, dry mouth and constipation. Diarrhoea and vomiting are seen less often. Insomia, dizziness, somnolence, and sweating are also seen, sexual dysfunction also occurs.

Combination therapy with venlafaxine and carbamazepine in depressive patients not responding to venlafaxine represents an interesting augimentation strategy by its efficacy, tolerance and absence of pharmacokinetic modification (Ciusani *et al.*, 2004).

Among other treatments that may successfully improve clinical response in non-responders, lithium augmentation appears to be more promising stategy (Bauer and Dopfmer, 1999). The anticonvulsant, valproate (Balfour and Bryson, 1994) have also shown efficacy as augmenting agents in patients not responding to TCAs or SSRIs.

2.4.5 Tetracyclic Antidepressants

Maprotiline has been shown to be an equally effective antidepressant that does not offer any clinically important difference from other TCAs. Seizures are about three times more likely with maprotiline than TCAs at higher doses. Compared to TCAs, maprotiline overdose involve more seizures, more delirium, and less cardiac arrythmias (Stimmel, 1980; Coccaro and Siever, 1985).

Maprotiline is a selective NE uptake inhibitor. It has less sedating effect than mianserin and is more epileptogenic than any other antidepressant (van Boxtel and Buitenhuis, 2000) and shows considerable cardiotoxicity when taken in overdose.

2.4.6 Dibenzoxazepines

Amoxapine is unique among antidepressants since it possesses some antipsychotic activity and may cause extrapyramidal effects and tardive dyskinesia because of its hydroxy metabolite's dopamine blocking activity. Although amoxapine could be considered an option for psychotic depression, it suffers from the disadvantage that the amount of antipsychotic activity is not predictable, and as a "fixed combination" product, there is not the needed flexibility of dosage titration of each drug, nor the ability to discontinue the antipsychotic and continue treatment with the antidepressant. For nonpsychotic depressions, amoxapine is not an appropriate choice, since it is the only antidepressant reputed to cause extrapyramidal effects, tardive dyskinesia and neuroleptic malignant syndrome. Amoxapine is less potent than most TCAs, meaning that the daily dosage must be higher. Amoxapine toxicity has fewer cardiac effects, but carries an increased risk of renal failure, seizures, and irreversible neurologic damage (Madakasira, 1989; Coccaro and Siever, 1985; Lydiard and Gelenberg,

1981). Amoxapine is similar to maprotiline in that it is a selective NE uptake inhibitor. It shares most of the properties of the TCAs.

2.4.7 Phenylpiperazines.

Trazodone was the first of the newer antidepressant drugs to offer several significant advantages over TCAs. Trazodone is one half as potent as TCAs such as amitriptyline and imipramine, meaning trazodone must be given in daily doses of 200 – 600mg. Many clinicians often abandon trazodone at doses of 200 – 300mg, claiming it to be ineffective (Bryant *et al*., 1990).

Trazodone does not have an appreciable effect on the re-uptake of the neurotransmitters dopamine or NE. It is a weak inhibitor of serotonin re-uptake but is a potent agonist of the serotonin 5-HT_2 receptor. Nefazodone and trazodone have ability to block 5-HT_1 presynaptic autoreceptors, and thereby, increase serotonin release (Howland and Mycek, 2006).

Trazodone commonly causes orthostatic hypotension, but administration of dosing after meals usually minimizes this effect. Trazodone is sedating probably because of its potent H_1 blocking activity, and some patients can not tolerate the sedation at daily dose of 400 - 600mg. In doses of 100 - 400mg daily, it can be given in a single daily bedtime dose with no loss of efficacy and less daytime sedation (Fabre, 1990).

Trazodone has no anticholinergic (antimuscarinic) effects, making it a useful consideration for patients who must avoid anticholinergic effects. Trazodone has no effect on cardiac conduction in contrast to TCAs that show cardiac conduction. Trazodone is safer in patients with angina and cardiac conduction defects. This lack of effect in cardiac conduction means

trazodone has no antiarrythmic effect, however, and there are case reports of some ventricular arrythmias being aggravated by trazodone (Coccaro and Siever, 1985; Georgotas *et al,* 1982).

Trazodone has also been associated with causing priapism. Priapism has been reported with many drugs, most commonly with phenothiazine antipsychotics, antihypertensives (mostly prazosin), as well as trazodone (Thompson *et al*., 1990). Case reports of priapism equivalent in females such as clitoral enlargement and increased libido in women receiving trazodone have been reported (Thompson *et al*., 1990; Gartrell, 1986).

Trazodone has become a commonly used hypnotic drug alone, as well as an adjunctive hypnotic for patients with persistent insomnia while taking fluoxetine or bupropion (Nierenberg *et al*., 1994a). There is little support however, for trazodone's use as a SSRI potentiator of efficacy despite such widespread clinical use (Nierenberg *et al*., 1992b).

2.4.8 Monocyclic Aminoketone

Bupropion is a monocyclic antidepressant, unique as a mild dopamine uptake inhibitor with no direct effect on NE, 5-HT, or MAO and essentially devoid of anticholinergic, antihistaminic and adrenergic effects (Stimmel, 1996c). An active metabolite is reported to block NE re-uptake.

Bupropion produces no clinically significant effect in cardiac conduction, orthostatic hypotension, or sexual function. It has minimal anticholinergic effect and causes little or no weight gain.

Frequent adverse effects include insomnia, agitation, headache and nausea (Preskorn and Othmer, 1984). Its lack of sedation and its activating effect may be advantageous for patients with decreased psychomotor activity and lethargy. Disadvantages of bupropion include seizures and the inability to dose once daily (Davidson, 1989). Bupropion is contraindicated in patients with psychotic disorders since its dopamine agonist effect may increase psychotic symptoms.

2.4.9 Lithium Salts

Lithium salts are used prophylactically for treating manic – depressive patients and in the treatment of manic episodes. They are also effective in treating sixty to eighty percent of patients exhibiting mania and hypomania.

Although many cellular processes are altered by treatment with lithium salts, the mode of action is unknown. Lithium is believed to attenuate signalling via receptors coupled to the phosphatidylinositol bisphosphate (PIP) second messenger system. Lithium interferes with the re-synthesis (recycling) of PIP, leading to its relative depletion in neuronal membranes of the CNS. PIP_2 levels in peripheral membranes are unaffected by lithium.

Lithium is given orally, and the ion is excreted by the kidney. Lithium salts are very toxic. Their safety factor and therapeutic index are extremely low – comparable to those of digitalis. Adverse effects include ataxia, tremors, confusion and convulsions. Dry mouth, polyuria and polydipsia are frequent complaints. The diabetes insipidus that results from taking lithium can be treated with amiloride. Thyroid function may be decreased and should be monitored. Lithium causes no noticeable effect in normal individuals. It is not a sedative, euphoriant or depressant (Howland and Mycek, 2006).

The antiepileptic drugs carbamazepine and valproic acid have been shown to alleviate some of the symptoms of mania (Balfour and Bryson, 1994).

2.5 ANTIEPILEPTICS

The various drugs or drug groups that are used for the treatment of epilepsy do not have the same indications and are not interchangeable. For different manifestations of the diseases, partial seizures, generalized seizures, either petit mal (absences) or grand mal (tonic- clonic seizures), different agents are considered to be drugs of first choice, dependent on differences in efficacy and tolerance.

Phenobarbital, primidone, phenytoin and also carbamazepine are inducers of cytochrome P450 enzymes and in combination the effects of substrates for these enzymes can be diminished. The therapeutic index of these agents is narrow and serum monitoring has to be performed on a regular basis. Antiepileptics are CNS depressants and patients should be warned for sedation. Antiepileptic drugs (AEDs) are grouped into the following classes.

2.5.1 Barbiturates and Derivatives

Barbiturates are administrered orally as anticonvulsants and intravenous administrations are used for anaesthetic purposes. Because of their low therapeutic index and their potential for abuse, barbiturates are no longer used as sedative-hypnotic agents and have been largely replaced for this indication by the safer BZDs.

Barbiturates bind to GABA channels to enhance Cl- transport and increase conductance at cl^- channels (van Boxtel and Buitenhuis, 2001). Their common pharmacological activity

consists of reversible depression of all excitable tissues (i.e. nervous tissues), but also, although, to a lesser extent, skeletal muscles, smooth muscles and cardiac muscle. In the CNS, a concentration dependent continuum of depression occurs, from mild sedation to deep anaesthesia. The effects in sleep are not too much different from normal sleep patterns. Pain perception is not influenced until unconsciousness is reached and small doses of barbiturates even produce hyperalgesic effects. Phenobarbital is considered to have the most selective anticuvulsant activity.

A wide range of drugs can interact with the barbiturates. Additive effects are seen with other sedatives. The metabolism of other drugs can be induced or the metabolism of the barbiturate can be diminished by cytochrome P450 inhibitors. The most frequent adverse reactions are those that are an extension of the therapeutic effects such as extended CNS depression manifesting itself as distortions in mood and impaired judgement and fine motor skills as well as intellectual performance as long as a day after usage (Dreifuss and Langer, 1988). Excitement and other paradoxical reactions can occur in the elderly but also in young children. Barbiturates generally increase the synthesis of porphyrin and intermittent porphyria is therefore an absolute contraindication for their use.

Other serious adverse events are the frequent occurrence of abuse and dependence. At toxic levels sometimes depressed cardiovascular tone will occur. In lethal barbiturate intoxications death is caused by central respiratory depression.

Phenobarbital is still used for the management of partial seizures, generalized tonic clonic seizures and for the control of status epilepticus. However, becaue of its low therapeutic index and the possibility of dependence, phenobarbital has largely been displaced by other

anticonvulsants. If given together with sodium valproate, the metabolism of phenobarbital may be inhibited while in combination with carbamazepine, the serum concentrations of carbamazepine will be reduced due to enzyme induction by Phenobarbital.

Primidone is another second line barbiturate used orally to control tonic-clonic and partial seizures. It is a prodrug as it is metabolized to Phenobarbital and phenylethylmalonamide (PEMA). However, both the parentcompound as well as the metabilies have antiseizure activity. Its use is more difficult to monitor and adverse effects occur even more frequently than with Phenobarbital.

2.5.2 Hydantoin Derivatives

Phenytoin is the only agent from this group that is frequently used. Its main indications are prophyllaxis of generalized tonic clonic, and complex and simple partial seizures. It is also used for the control of status epilepticus after initial treatment with diazepam.

It prolongs inactivation of Na+ channels in the CNS and increase the depolarization threshold. Phenytoin is able to diminish the generalisation of seizures but does not stop seizure focus activity.

Because phenytoin is metabolised in the liver and is also a liver enzyme inducer, many drug interactions have been described. For example, the efficacy of oral contraceptives and of oral anticoagulants may be decreased. Enzyme inducers such as barbiturates decrease its potency. Also related to induction of enzyme activity is interference with vitamin D metabolism which may cause osteomalacia and rickets (van Boxtel and Buitenhuis, 2001).

Dose and concentration dependent adverse effects are nausea, vomiting, tremor, confusion, headache and dizziness, nystagmus and ataxia. Long term use frequently induces hyperplasia of the gums. Skin rashes are seen in up to 10% of the patients. Serious, most probably pseudo, allergic skin eruptions can also occur.

Ethotoin has appeared to be of some value in the treatment of partial as well as generalized tonic-clonic seizures and to be relatively free of the typical adverse effects of phenytoin. Because of its low efficacy, it is rarely used and then only in combination with other agents.

2.5.3 Benzodiazepine derivatives

A few BZDs are frequently used as anticonvulsants and clonazepam is exclusively used for its anticonvulsant activity. It has shown efficacy in all forms of epilepsy but it is mainly used for the management of myoclonic and atonic-akinetic seizures in children. It may also be used as an alternative to diazepam in the emergency treatment of status epilepticus. It has a wide therapeutic range in comparison to other anticonvulsants.

Adverse reactions such as fatigue, drowsiness and ataxia are mainly related to its sedative activity. In the elderly a greater sensitivity to CNS effects can be expected. Bronchial hypersecretion may cause respiratory problems in infants and small children.

Intravenously administered diazepam is first line therapy for status epilepticus. However, there is a serious risk for severe depression, hypotension, tachycardia and cardiac arrest.

2.5.4 Succinimide Derivatives

Ethosuximide and mesuximide are succinimides. Ethosuximide is the agent of first choice for the management of absence (petit mal) seizures. It inhibits low-threshold t-type Ca^{2+} currents in the thalamus.

As it may precipitate grand-mal seizures it is frequently given together with a barbiturate or with phenytoin to prevent that. Enzyme inducers will enhance the metabolism of ethosuximide and reduce its efficacy while its depressant action will be enhanced by other sedatives.

Frequently occurring adverse effects include sedation, and gastrointestinal disturbances such as nausea and vomiting. Rarely, blood dyscrasias, including agranulocytosis and pancytopenia are seen as well as serious skin reactions including Stevens – Johnson syndrome.

2.5.5 Carboxamide derivatives

Carbamazepine is a tricyclic iminostilbene derivative and structurally related to the TCAs. It is used as a first line agent for the management of generalized tonic-clonic epilepsy. It is also highly effective for the management of complex partial seizures (Mattson *et al*., 1992) but has no efficacy in patients with absence seizures or atonic seizures. In epilepsy, it supposedly has the same mechanism of action as phenytoin.

Frequently occurring adverse effects are sedation, dry mouth, dizziness and gastrointestinal disturbances. Photosensitivity reactions, urticaria and Stevens-Johnson syndrome have been described. The elderly are more prone to mental confusion, cardiac abnormalities and

problem due to inappropriate ADH secretion. Leukopenia and aplastic anaemia have also been reported (Hart and Easton, 1982). Oxcarbazepine is a derivative of carbamazepine, and although its precise mechanism of action is unknown, it has similar properties as carbamazepine and is also used for the treatment of primary generalized tonic-clonic seizures and partial seizures.

Adverse effects are also similar to those of carbamazepine. However, the drug interaction profile is different as oxcarbazepine has hardly any enzyme-inducing capacity.

2.5.6 Miscellaneous Antiepileptics

Valproic acid is a fatty acid derivative which is used for the management of absence and control of generalized tonic-clonic seizures (Mattson, 1989). Multiple mechanisms of action have been proposed. It prolongs N^{a+} channel inactivation which could explain its effectiveness against grand mal seizures.

However, also inhibition of T-type Ca^{2+} channels has been postulated. Inhibition of GABA transaminase would increase GABA levels, but as this only occurs at high doses its importance is under debate. Valproic acid is metabolised in the liver and excreted in the urine mainly as glucuronide conjugates. Valproic acid is not a hepatic enzyme inducer.

Valproate causes a dose-related reduction in platelet count and impairment of platelet function leading to an increase in bleeding time (Gidal *et al*, 1994). Frequently occurring adverse effects are gastrointestinal complaints and dose-related CNS effects including fatigue, sedation, ataxia, dysarthria and other symptoms of inco-ordination. Rare, but potentially dangerous reactions are bone marrow depression and pancreatitis. The risk of

serious and potentially fatal hepatotoxicity is greater in children under 2 years. Cognitive and behavioural AEs are less common (Dreifuss and Langer, 1988). Trimethadione is an effective agent in the treatment of absence seizures. Trimethadione is N-demethylated to dimethadione and dimethadione inhibits T-type Ca^{2+} currents in the thalamus. Because of its potential for serous toxicity, trimethadione is only used in patients who do not respond to or do not tolerate other agents.

Lamotrigine is a phenyltriazine derivative and it is used for partial seizures, mostly in combination with other drugs. Lamotrigine has been shown to increase valproate concentration by 25% in healthy volunteers (Lamictal prod. Info., 1994). It is thought to act by blocking voltage – dependent Na+ channels and by inhibiting the release of the excitatory neurotransmitter, glutamate. The most common adverse effects are dizziness, ataxia, blurred vision and nousea and vomiting. Preliminary evidence suggest that the drug also has activity against primary generalized epilepsies (including juvenile myoclonic epilepsy) and the Lennox-Gastaut syndrome and that it is effective as monotherapy (Harden, 1994). About 10% of patients who received lamotrigine developed a skin rash, and it was the most common reason for drug discontinuation – 3% (Lamictal Prod. Info., 1994).

Gabapentin is a new antiepileptic with efficacy in partial seizures with and without secondary generalization. It is also mainly used in addition to other antiseizure drugs. The use of gabapentin in mixed, seizure disorders that include absence seizure is contraindicated as these may be exacerbated.

Gabapentin, structurally related to the neurotransmitter GABA does not alter GABA synthesis, degradation, or uptake or interact with GABA receptors (Harden, 1994). It is not

bound to plasma proteins, and does not induce hepatic enzymes. No interactions with other antienpleptics have been described. Adverse effects are somnolence, dizziness, ataxia and fatigue but some tolerance to these effects occurs within a few weeks.

Vigabatrin is a new antiepileptic for use in epilepsy unresponsinve to other therapy. It is an irreversible inhibitor of GABA – transaminase, the enzyme responsible for inactivation of the neurotransmitter GABA (Harden, 1994) and it has shown efficacy against partial and secondarily generalized seizures. The principal unwanted effects are psychiatric disorders, including depression and psychosis, in a small number of patients.

Felbamate, a new anticonvulsant, has beneficial effects in partial and secondarily generalized seizures. It can reduce symptoms in Lennox-Gastaut syndrome. However, development of plastic anaemia and hepatic adverse effects (Felbatol Prod. Inf., 1994) reduced its usefulness and Lennox-Gastaut syndrome is considered to be its only indication.

2.6 **Medicinal Plants with Central Nervous System Activity**

The World Health Organization (WHO) estimated that almost 80% of the people living in developing countries exclusively use traditional medicines (Eloff, 1998). Earlier the Alma-ata declaration of 1978 provided for the inclusion of herbal medicines of proven efficacy and safety into the healthcare programme of developing countries. In the developed countries more recently, significant interest has been shown by consumers in the use of herbal medicines as products from natural origin. Consequently, the WHO has advocated for the proper identification, sensible exploitation, scientific evaluation and appropriate utilization of herbal medicines (Chindo, 2006).

Quite a number of medicinal plants are proving to be important sources of therapeutic agents. The therapeutic benefits of traditional remedies depend upon a combination of constituents (Amos *et al.,* 2001). For instance, alkaloids and saponins have been reported to show potent sedative activity, they have also been shown to have antagonistic activity in amphetamine and known to inhibit spontaneous motor activity in mice (Wagner *et al.,* 1983; Dubois *et al.,* 1986; Taesotikul *et al.,* 1998). Woo *et al.,* (1980) also reported the sedative (CNS depressant properties) of saponins and flavonoids isolated from *zizyphus* seeds i.e. *zizyphus jujuba.*

More recently, the anticonvulsant effects of the following plants have been reported. *Dalbergia saxatilis* (Yemitan *et al.,* 2001; Yemitan and Adeyemi, 2005); *Magnolia grandiflora* (Bastidas Ramirez *et al.,* 1998) ; *Cyperus articulatus* (Bum *et al.,* 2001) ; *Delphinium denudatum* (Raza *et al.,* 2001) among others.

Similarly, the antidepressant activities of the *areca cataechu* (Dar *et al.,* 1997) and the anxiolytic and muscle relaxant properties of *Dalbergia saxatilis* (Yemitan and Adeyemi, 2003) have been reported. Musa *et al.,* (2006) and Adzu *et al.,* (2001) also reported the CNS depressant effects of *ficus thoningii* and *ziziphus spina-christi* respectively.

Randia nilotica as was stated earlier is a lowland shrub widespread in Northern Nigeria, the Cameroon, Sudan and East Africa (Lemmich *et al.,* 1999). It is popularly known as ‘tsibra’, ‘barbaji’ or ‘gial-goti’ among the Hausa-Fulani speaking communities of Northern-Nigeria (Dalziel, 1937). Folkloric uses include the use of the decoction of the plant orally for treatment of mental breakdown and convulsions (Chhabra *et al.,* 1991). Also the use of the dried root in Tanzania against epilepsy and madness has been reported (Hedberg *et al.,* 1983).

Branches of the plant are whitish, pubescent, armed with sharp spines 1-3 cm long. Spines are usually solitary and alternate, but sometimes in unequal pairs. Leaves and flowers are on much abbreviated lateral branchlets borne in pairs just below the spines. The leaves are obovate, cuneate at base, rounded at apex, 2 – 7 cm long, 1– 3 cm broad, shortly pubescent especially beneath, with 3 – 4 main lateral nerves on each side of midrib. Flowers are solitary or paired, terminating the lateral branchlets. Pedicels are 5 – 10 mm. long, calyx about 7 mm long, shortly toothed, glabrous outside with corolla about twice as long as the calyx, villous outside. Fruits are ellipsoid, 1.8 – 2 cm long, 1 -1.5 cm broad, glabrous and two – celled (Hutchinson and Dalziel, 1963).

The plant is believed to contain active substances with potential for use in treatment of neuropsychiatric and neurological disorders.



Plate 1: ***Randia nilotica*** **Stapf. in its natural habitat.**

CHAPTER THREE

3.0 Materials and Methods

3.1 Experimental Sites

Most of the experiments conducted were in the Laboratories of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Other experiments were carried out in the Laboratories of the Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Research and Development, Idu, Abuja, Nigeria.

3.2 Plant Material

3.2.1 Plant Collection

Three batches of the plant material (leaves, stem and root barks) were collected. The first one was from Gusau, Zamfara State while the second was from Malumfashi, Katsina State and the last batch was from Zaria, Kaduna State-all in Northern Nigeria. The collection in each case was done between the months of August and October. Parts of the plant collected were the leaves, stem bark and root bark. Each batch was identified and authenticated by Mall. Musa Shehu and Mall. Umar Shehu Gallah, taxonomists with the Department of Biological Sciences, Ahmadu Bello University, Zaria –Nigeria. These were compared with a voucher specimen (No. 2867), deposited at the Herbarial Section of the same Department.

3.2.2 Preparation of the Plant Extracts

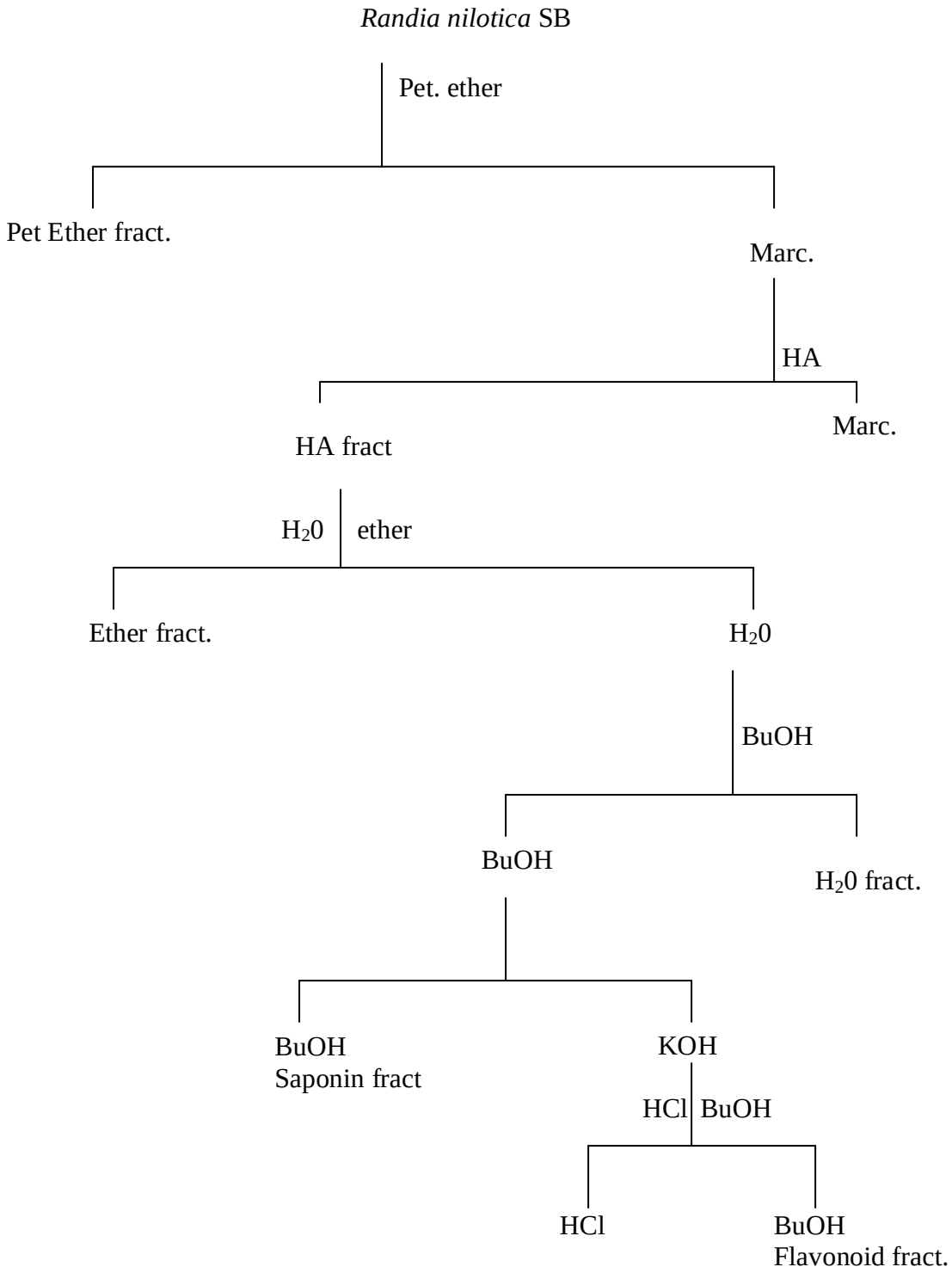
The stem bark and root bark were carefully removed, washed and cut into pieces. The stembark, rootbark and leaves were air dried for 7-10 days under room temperature, and powdered using a pestle and mortar and then sieved. The powdered material was macerated in hydroalcoholic solution (Elthanol-70% and water – 30%) in the ratio 1:2.5 (powdered

material: hydroalcoholic solution), with occasional shaking for 24 hrs and then filtered. The filterate was evaporated to dryness in vacuo at 40^0C and stored in a dessicator. The extracts were investigated to determine which portion possesses more CNS activity using the diazepam – induced sleep in mice.

3.2.3 Separation of Extract of *Randia nilotica* Stembark

The method described by Woo *et al.,* (1980) was followed. The scheme below shows the procedure of extraction and fractionation. The method involves defattening initially with petroleum ether followed by extraction with hydroalcoholic solution. Polar compounds were further removed by dissolving the hydroalcoholic extract in diethylether solution with subsequent addition of water. Butanol was then added to the water residue and the mixture shaken vigorously. The two distinct layers were then separated and 1% potassium hydroxide (KOH) solution was added to the butanolic residue and gently shaken. The butanolic portion contains the saponins while the KOH solution when acidified with concentrated hydrochloric acid gave the flavonoid fraction.

Fig. 1: Successive extraction of *Randia nilotica* stembark



The HA extract was fractionated as shown above and each fraction was first subjected to phytochemical screening and then investigated for CNS activity. Column chromatographic separation of the saponin fraction was also carried out on Sephadex with methanol as

feluent.The subfractions (SF_1-SF_5) so obtained were subjected to thin layer chromatograpy(TLC) using silica-coated plates.The solvent medium used was Ethylacetate:Formic acid:Acetic acid:Water (10:1.1:1.1:2.6).

3.2.4 Phytochemical Screening

Phytochemical analysis of the extracts was performed according to the methods of Clarke (1975), and Trease and Evans (1989). The extract was screened for the presence or absence of alkaloids, cardiac glycosides, saponins, steroids, tannins, flavonoids, anthraquinones e.t.c. The fractions obtained through fractionation were also tested to ascertain their identity.

3.2.4.1 Test for Alkaloids

3 g of the hydroalcoholic stembark extract was dissolved in 50% of ethanol. The solution was mixed with 10 ml of 1% hydrochloric acid. 1ml portions of the solution were treated with few drops of the following reagents: Dragendorff's reagent (potassium Bismuth iodine solution) and Wagner's reagent (solution of iodine in potassium iodide). Deep brown-coloured precipitate and turbidity with Dragendorff's and Wagner's reagents is indicative of the presence of alkaloids in the extract.

3.2.4.2 Test for Tannins

3 g of the crude HA extract was boiled in 50 ml of distilled water for 3 mins on a hot plate. The mixture was filtered and the resultant filtrate used for the following test:

3.2.4.2 Lead Subacetate Test

A few drops of the previously diluted (1:4) filterate were mixed with lead subacetate solution and light brown – coloured precipitate indicate the presence pf tannins (Trease and Evans, 1989).

3.2.4.3 Test for Saponins (Froths Test)

A small quantity of the crude extract was mixed with 50ml of 95% ethanol and boiled. The mixture was filtered and 2.5ml; of the filterate was added to 10 ml of distilled water in a test tube. The test tube was stoppered and shaken vigorously for about 30 sec. It was then allowed to stand for half an hour. Persistent honey comb froth indicated the presence of saponins.

3.2.4.4 Test for Saponin Glycosides – Keller – Kiliani Test

1 g of the initial powdered material was boiled with 10ml of 70% alcohol for 2 – 3 min and filtered. To 5 ml of the filterate 10ml of water and 0.5 ml of strong solution of lead acetate were added and then shaken and filtered. Filtrate was shaken with 5ml of chloroform and allowed to separate. The chloroform was pipetted off and the solvent removed by gentle evaporation in a porcelain dish. The cooled residue was dissolved in 3ml of glacial acetic acid containing 2 ml of concentrated sulphuric acid. A reddish brown layer formed at the junction of the two liquids and the upper layer slowly becomes bluish – green, darkening with standing (Trease and Evans, 1989).

3.2.4.5 Test for Triterpenes – Libermann- Butcher Test

To 3 g of the initial powdered material was added to 10 ml of chloroform. The solution was shaken vigorously and then filtered. To 5 ml of the filtrate was added equal volume of acetic anhydride and then concentrated sulphuric acid by the side of the test tube. The appearance of

a brown ring at interface and greenish colouration of the chloroform layer indicated the presence of triterpenoidal saponins.

3.2.4.6 Test for Anthraquinones – Modified Borntrager's Test

The powdered material was first extracted with benzene. The benzene extract was then vigorously shaken with dilute ammonia. A cherry red colour at the ammoniacal layer indicate presence of anthraquinones (Trease and Evans, 1989).

3.2.4.7 Test for Flavonoids – Shinoder Test

2 g of the powdered material was macerated in 50% alcohol and then filtered. To 5 ml of the filtrate were added pieces of magnesium metal and then drops of 1% hydrochloric acid. The appearance of bubbles and reddish to yellow colour indicated the presence of flavonoids.

3.3 Animals

All the experiments performed on laboratory animals in this study followed the "principles of laboratory animal care" (NIH publication, 1985).

Swiss albino mice (18-25g), wistar rats (180-250g) of either sex maintained at the Animal House of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria were used for the study. In addition, black ranger cockerels, obtained from the National Animal Production and Research Institute, Shika, Zaria were also used. They were housed under standard conditions of temperatures (25 ± 2^0C) and light. They were fed on standard diet and water *ad libitum.*

3.3.1 Drug Solutions

3.2.1.1 Preparation of Drug Solutions

i) The hydroalcoholic extracts, saponin fraction, flavonoid fraction, aqueous fraction, d-amphetamine sulphate (Sigma Chemical Comp USA), pentobarbitone sodium (Sigma Chem. Comp. USA), strychnine (Sigma Chem. Comp. USA), Imipramine, Pentylenetetrazole (Sigma Chem. Comp. USA), were prepared by dissolving the powder in deionized water prior to administration.

ii) Diazepam ('Tranzite' – Ningbo Pharm Factory), chlorpromazine and phenobarbitone were supplied in ampoules and appropriate dilutions were made with deionised water. The drug solutions were usually prepared freshly for each day's experiment to maintain stability of the drugs used. The solutions were kept in air-tight, amber-coloured containers and stored in the refrigerator ready for use.

3.3.3.2 Routes of Drug Administration

The HA stembark extract saponin fraction, d-amphetamine chlorpromazine, diazepam, phenobarbitone and pentobarbitone were administered intraperitoneally while pentylenetetrazole and strychnine were administered subcutaneously.

3.3.3.3 Volume of Injected Drug Solutions

The volume of drug solution administered to each mouse, rat or chick was maintained at a constant proportion irrespective of the dose.

3.4 Pharmacological Studies

3.4.1 Acute Toxicity Studies

The acute toxicity of the crude extracts was investigated in mice by intraperitoneal injection. The method used was as described by Lorke (1983).

The study was carried out in two phases. In the intitial phase, the range of doses producing the toxic effects was established. Three groups of 3 mice each were used. The first group received extract at a dose of 10 mg/kg i.p., while the second and third groups received extract at doses of 100 and 1000 mg/kg respectively. The animals were observed for signs of toxicity and death within 24 hrs.

In the second phase, three groups of 1 mouse each was used. Specific doses were administered which depended on the result of the first phase. The final LD_{50} (dose that is responsible for the death of 50% of an animal population) value was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, that is the geometric mean of consecutive doses for which 0 and 100% survival rates were recorded.

All the extracts including stembark, rootbark, leaf and the saponin, flavonoid and aqueous fractions were subjected to this test to determine their LD_{50}.

3.4.2 Diazepam Induced Sleep in Mice

To compare the CNS depressant effects of various crude extracts and fractions, the method described by Rakotonirina *et al.* (2001) was used. Sleep potentiating effects of the extracts and fractions were studied in a group of mice that received diazepam at a dose of 30 mg/kg i.p., 30 min after administration of extract or fractions. There were 6 mice in each group of

four groups. The criteria for sleep was considered to be loss of righting reflex (Miya *et al.*, 1973) and sleeping time was therefore measured as the time between disappearance and recovery of the righting reflex (Fujimori and Cobb, 1965; Soulimani *et al.*, 2001).

The doses of the HA stem bark and root bark extracts used were 5, 10 and 20 mg/kg body weight, while those of the leaf extract were 100, 200 and 400 mg/kg. The doses of saponin fraction used were 0.5, 1.0, and 2.0 mg/kg while those of the aqueous and flavonoid fractions were 20, 40 and 80 mg/kg in each case. 0.125, 0.25 and 0.5 mg/kg doses were used in the case of subfractions. The doses were determined from the calculated LD_{50} values (20% and below) of the extracts.

3.4.3 Other Behavioural Studies

3.4.3.1 Hole – Board Test

The influence of the extract on exploratory activity of mice was determined using the hole-board test (File and Wardill, 1975) as modified by Yemitan *et al.*, (2001). The apparatus used consists of a white wooden board (40 cm. x 40 cm.) with four equidistant holes (1 cm. diameter x 2 cm. depth).

Five groups of 6 mice each were used. Each mouse was placed at one corner of the board and the animal moved about and dipped its head into the holes indicating exploratory behaviour. The number of dips in 7.5 minutes was recorded. The test was carried out 30 min. after i.p. treatment with the extract at doses of 5, 10 and 20 mg/kg. Normal saline (1 ml/kg) and diazepam (1mg/kg) were used as negative and positive controls respectively.This test was repeated using the saponin fraction at doses of 0.5, 1.0 and 2.0 mg/kg body wt.

3.4.3.2 Pentobarbitone induced sleep test

The method is as described above in 3.4.2. Pentobarbitone sodium 20 mg/kg was used as the treatment agent 30 min after pre-treatment with either RNBE or SFRN. Diazepam (1 mg/kg) was used as control.

3.4.3.3 Test for Motor Co-ordination (Rota-rod Test) in Mice

The method used for the assessment for locomotor (forced motor) activity in mice was as described by Kinnard and Kar (1957), Krsiak (1975); Ozturk *et al.*, (1996).

A rota-rod treadmill device (Ugo Basile N^0 7600, Varese, Italy) was used for this purpose. Mice were trained to remain on slowly – moving (16 – revolutions/minute) rods of 5 cm diameter for 150 sec. by walking. They were divided into 4 groups of 6 mice each. Mice in the first group served as control, while those in the second, third and fourth groups received stem bark extracts of *R. nilotioca* at doses of 5, 10 and 20 mg/kg body wt. i.p. respectively. Thirty minutes after receiving the injection, animals were placed on the rod at intervals of 30 min up to 2 hrs. If an animal failed more than once to remain on the rod for 3 mins., the test was considered positive, meaning that there was a lack of co-ordination (Fujimori and Cobb, 1965).

The same procedure was repeated using the saponin fraction at doses of 0.5, 1.0 and 2.0 mg/kg i.p.

3.4.3.4 Mouse Beam Walking Assay

The method of Stanley *et al.* (2005) was used. Mice were trained to walk from a start platform along a ruler (80 cm long, 3cm wide) elevated 30cm above the bench by metal

supports to a goal box (enclosed hamster house). Three trials were performed for each mouse, and were designed such that the mice tested would be aware that there was a goal box that could be reached. A ruler was used because the mouse found this easy to cross and, at the same time, it induced minimum anxiety.

Once the mice had been tested on the rotarod, they moved immediately to the beam test. The beam was made of wood, 8mm in diameter, 60 cm long and elevated 30 cm above the bench by metal supports.The mice were 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, with a maximum time of 60 sec. allowed on the beam. The measurements taken were time spent on beam before reaching the goal box, the number of foot slips (one or both hindlimbs slipped from the beam) and the number of falls.

3.4.3.5 Stereotyped Behavioural Studies in Mice

The method of Randrup and Munkvad (1967) and Ellinwood *et al.*, (1973) was followed for the stereotyped behavioural studies. The test was performed in five groups of six mice each. The first group received normal saline as control, while animals in the second, third and fourth groups, received the extracts at doses of 5, 10 and 20 mg/kg body wt. i.p. respectively. The animals in the fifth group were injected intraperitoneally with chlorpromazine (2 mg/kg) body weight. Thirty minutes after drug administration and saline, all the animals were injected with d-amphetamine (2 mg/kg). The signs of stereotype behaviour recorded include circling, jumping limb licking, sniffing and general locomotion.

Similarly, the saponin fraction was subjected to the experiment at doses of 0.5, 1.0 and 2.0 mg/kg body weight.

3.4.4 Anticonvulsant Studies

3.4.4.1 Electro-Shock Induced Convulsion Test

The methods of Swinyard and Kupferberg (1985) and of Browning (1992) were employed. The apparatus used was the Ugo Basile Electroconvulsive Machine (Model 1801) with corneal electrodes placed on the upper eyelid of the chicks after dipping them in normal saline. Day old chicks have an underdeveloped blood brain barrier thereby facilitating easy passage of drugs and current into the brain.

A current which induces tonic seizures in 80-90% of a control group of chicks (95 mA) was selected. The shock duration (0.8s), pulse width (0.8mm) and frequency (150Hz) determined, were maintained for all other groups. A second group of ten chicks was pretreated with phenobarbitone (20 mg/kg) i.p. 30 mins. later, they were subjected to electrical stimulation as in untreated controls. Test chicks were then pretreated in groups of ten with 5, 10 and 20 mg/kg of *R. nilotica* extract i.p., before being electrically shocked 30 mins later.

Results were recorded as either positive or negative depending on whether tonic hind limb extension was produced. The experiment was repeated with the saponin fraction at doses of 0.5, 1.0 and 2.0 mg/kg i.p. in mice (n=6).

3.4.4.2 Pentylene-tetrazole (PTZ) Induced Convulsion test in Mice

Mice were injected with a convulsive dose (CD_{90}), that is the dose that produces convulsions in about 90% of animals (90 mg/kg PTZ subcutaneous-ScPTZ) – Krall *et al.* (1978); Raza *et al.*, (2001). They were then observed for the presence or absence of threshold seizures (an episode of clonic seizure of at least 5 sec. duration), PTZ-induced seizures were further

characterised into different patterns, that is one or more generalised myoclonic body twitches, generalised body seizures with loss of the righting reflex, loss of righting reflex with tonic fore limb extension and loss of the righting reflex with tonic fore and hind limb extensions (Loscher *et al.*, 1991b).

Three groups of 6 mice each were pretreated with the extract at 5, 10 and 20 mg/kg body weight, respectively. A fourth group was pretreated with phenobarbitone 30 mg/kg body weight i.p. Another group was given normal saline 1ml/kg and served as control. Thirty minutes later, all the mice were injected with a convulsive dose (CD_{90}) of PTZ subcutaneously. They were then observed as described previously.

This experiment was repeated with the saponin fraction at doses of 0.5, 1.0 and 2.0 mg/kg body weight i.p.

3.4.4.3 **Strychnine (STN) – Induced Convulsion Test in Mice**

Mice were injected with a convulsive dose CD_{90} of strychnine (Krall *et al.* 1978; Raza *et al.*, 2001)- 1.2 mg/kg body weight. They were then observed for the presence or absence of convulsion. Abolition of tonic extensor jerks of the hind limbs was considered an indicator that the material could prevent STN-induced seizures (Porter *et al.*, 1984; Raza *et al.*, 2001).

Five groups of six mice each were used for the study. The first group received normal saline while three other groups received *R. nilotica* extract at 5, 10 and 20 mg/kg body weight respectively. The fifth group was injected i.p. with phenobarbitone 30 mg/kg). Thirty minutes later, all the mice were injected with a convulsive dose of STN and observed for 30 min for signs of convulsion.

3.4.5 Other Pharmacological Tests

3.4.5.1 Forced Swim Test in Rats

The study was carried out according to the method of Porsolt *et al.* (1978) as described by Krozcka *et al.* (2001). This test predicts antidepressant efficacy in human depression (Porsolt *et al.,* 1978; Porsolt *et al.,* 1977a). The rats were placed in plexiglass cylinders (height 40 cm, diameter 18 cm) containing 17 cm of water at 25^0C. The rats were removed and allowed to dry in a heated enclosure (32^0C) before being returned to their home cages after the 15 mins. pre-test swim in water.

They were divided into 4 treatment groups of 5 rats per group. Three groups received graded doses of the extract *R. nilotica* (5, 10, 20 mg/kg i.p) while the other group received normal saline as control. The extracts were administered three times at 24, 5 and 1 hr before the test. The rats were again placed in the cylinders 24 hr later. The duration of immobility in each rat was measured during a 5 min. test. A rat was judged to be immobile when it remained floating passively in the water.

3.4.5.2 Tail Suspension Test in Mice

This was carried out as described by Steru *et al.,* (1985) with modifications. Adult mice of either sex were randomly divided into four (4) groups of five (5) mice each. Three groups were administered graded doses of the extract *R. nilotica* (5, 10 and 20. mg/kg body weight i.p. respectively). Animals in the control group received normal saline. Thirty minutes, later, each mouse was suspended by the tail on the edge of a shelf 58 cm above a table top and the length of their immobility recorded during a 6 min period as described previously (Steru *et al.,* 1985), after discarding activity in the first 2 mins during which an animal tries to escape

(Jain *et al.*, 2003). A mouse is considered immobile only when it hung passively and remains motionless.

This procedure was repeated with the saponin fraction at doses of 0.5, 1.0 and 2.0 mg/kg body weight.

3.4.5.3 Electroencephalographic (EEG) Studies in Rats

The first step in EEG studies is implantation of the electrodes:electrocortical and electromyographic. Rats were anaesthesized using halothane and ketamine 100 mg/kg body weight. The rats were held firmly with earplugs on a stereotaxic apparatus and were surgically implanted with stainless steel screws (Grass Instruments) No. 00 (Spooner, 1965; Sokomba *et al.*, 1986). The sites of implantation include optic tectum (OT), Hyperstriatum (HS), and midbrain Reticular Formation (RF). The stereotaxic coordinates of sites of implantation were determined for each rat separately.

The surface locations of the brain areas were first marked before drilling the skull and implantation of electrodes. The implanted electrodes were held firmly in place using dental cement. The neck region was similarly implanted for electromyographic EMG recordings. The EMG electrodes were made of stainlless steel insect pins No. 00, which were bent at the tips into hooks and inserted proximally to the head into semispinalis capitis muscle on the left side of the dorso-lateral aspect of the rat.

The implanted electrodes were connected to the male connector as follows: monopolar electrodes from upper surface of the mouth, No.1; monopolar electrodes from the frontal cortex, No.2; bipolar electrodes from brain reticular formation (RF), No.4,5; two EMG

electrodes from the neck, No.6,7. After implantation process was completed, the rats were kept warm with an angle-poised lamp and allowed 24 h for full recovery from anaesthesia before EEG recording. Before using the EEG machine, it was calibrated and the control settings selected.

The electrodes of the implanted rat were attached to the male connector and hooked into the female connector. The male connector was positioned in a screened cage to avoid interference. The rat was allowed in the screened cage for a period of one hour to settle down in the new environment before EEG recording.

The recording of both the EEG and EMG were done with grass polygraph, Model 79D. The chart speed during normal recording was 2.5 mm/s. However, at appropriate times, the speed was changed to 25 mm/s for at least 10 seconds to facilitate analysis of voltage and peaks. Each time, the electrical activity was recorded for at least 30 minutes to allow for stabilisation and control before injecting the test compound. The crude hydroalcoholic stembark extract (5, 10 and 20 mg/kg) and crude saponins (0.5,1.0 and 2.0 mg/kg) as well as SF_1 subfraction (0.25 mg/kg) were then injected into the rats. Activity was recorded for 2 hours and compared with control.

CHAPTER FOUR

4.0 RESULTS

4.1 Plant Extract

The results from the three batches of the plant collected from different areas in Northern Nigeria namely Gusau, Malumfashi and Zaria were seen to be similar.

The various extracts obtained were weighed and their respective percent yield determined as shown in table 4.1

Table 4.1: Weight (gm) of starting materials and respective percentage yield (w/w) of extracts of *Randia nilotica*.

Plant part	Yeild (powder) gm.	Percent yield (w/w)
Root bark	**8.10 (50)**	**16.2**
Stem bark	**9.20 (50)**	**18.4**
Leaves	**15.46 (100)**	**15.5**

4.1.1 Separation of Hydroalcoholic (HA) Stembark extracts of *Randia nilotica.*

Successive extraction of the stembark extract yielded saponins , flavonoids , Aqueous extract, ether fraction and petroleum ether fraction .The percentage yield for these fractions was also determined (Table 4.2).

Table 4.2: Yield of various fractions obtained from successive extraction of the HA stem bark extract of *Randia nilotica*.

Fractions	Yeild (gm)	Percent (w/w)
Flavonoids	**0.86**	**0.86**
Saponins	**1.00**	**1.00**
Aqueous	**3.02**	**3.02**
Diethyl ether	**0.60**	**0.60**
Petroleum ether	**_**	**traces**

4.1.2 Result of Phytochemical Screening of Stembark Extract

Test for alkaloids: No red precipitate with Dragendorff's and no brown precipitate with Meyer's and Wagner's reagents.This indicated absence of alkaloids.

Test for tannins: Presence of blue colouration in the ferric chloride test as well as light brown coloured precipitate in the lead subacetate test indicated the presence of tannins.

Frothing test for saponins: Shaking resulted into the formation of copious froth that persisted for over 15 minutes. This indicated the presence of saponins.

Test for cardiac glycosides: Appearance of bluish-green colour within 5 min from addition of sulphuric acid in the Keller-Kiliani test indicated presence of cardiac glycosides.

Test for sterols/terpenes: At contact zone of sulphuric acid deposited at bottom of the tube with the solution,a violet ring was formed and green in the supernatant liquid.This indicated presence of triterpenes.

Borntragers test: On heating no change in colouration was observed.This confirms absence of coumarins anthraquinones.

Test for flavonoids: Orange colouration was observed in the Shinoda test.This indicated the presence of flavonoidal aglycone.

A summary of results of the preliminary Phytochemical screening of the extract is presented (Table 4.3).

Table 4.3: Phytochemical components of *Randia nilotica* stem bark extract.

Compound group	Present/Absent
Alkaloids	–
Tannins	+
Saponins	+
Cardiac glycosides	+
Sterols/Terpenes	+
Anthraquinones	–
Flavonoids	+

Key:

Present +

Absent -

The saponins obtained from successive extraction of the crude extract, tested positive to Libermann-Butcher and Froths tests. They also tested negative to shinoda and ferric chloride tests indicating the absence of flavonoids in them.

4.1.3 Column Chromatographic Separation of Saponin Fraction of *Randia nilotica*

The saponin fraction was subjected to column chromatography which yielded 19 subfractions. TLC analysis allowed pooling of these subfractions into 5 (Table 4.4).

Table 4.4: Eluates obtained from column chromatography of saponin fraction of *Randia nilotica*.

Eluates	Number of spots	Code
1-4	**2**	**SF_1**
5-9	**4**	**SF_2**
10-11	**3**	**SF_3**
12-14	**2**	**SF_4**
15-19	**0**	**SF_5**

4.2 Pharmacological Studies

4.2.1 Acute Toxicity Studies

Signs and symptoms observed in test animals injected with both crude extracts and fractions included piloerection, reduced mobility, respiratory embarrassment including gasping with eventual immobility and death.

The intraperitoneal LD_{50} of the crude HA extracts of the leaves, stem bark and root bark were calculated as well as that for the aqueous fraction, flavonoids and saponins in mice and rats (Table 4.5).

Table 4.5: LD_{50} values for various extracts and fractions of *Randia nilotica* stem bark

Test compound	Animal model	LD_{50} value(mg/kg)
Leaf extract	**mice**	**2154.1**
Rootbark ext.	**mice**	**282.8**
Stembark ext.	**mice**	**282.8**
Stembark ext.	**rats**	**471.2**
Saponin fraction	**mice**	**11.1**
Saponin fract.	**rats**	**70.7**
Flavonoid fract.	**mice**	**471.2**
Aqueous fract.	**mice**	**471.2**

4.2.2 Effect of the Crude Extracts of *Randia nilotica* stem bark on Diazepam-Induced Sleep in Mice

The crude HA extract of the stem bark showed a dose-dependent decrease and a dose-dependent increase in onset and duration of sleep respectively. The decrease in onset was significant ($p < 0.05$) at 10 and 20 mg/kg (3.2±0.2 min. each) compared to normal saline (5.2±0.7 min.). Significant difference in sleep duration ($p < 0.05$) also exists at 5 mg/kg (51.8±13.1 min.) and ($p <0.001$) at 10 and 20 mg/kg body weight (166.2±14.8 and 165.8±13.0 min. respectively) compared to normal saline (35.2±6.1 min.). The difference between groups is also significant ($p<0.001$) - Fig. 4.1

The root bark extract however, did not exhibit any significant difference in onset of sleep at all doses tested with control .A significant difference in sleep duration ($p <0.05$) between normal saline (35.2±6.1 min.) and extract at 20 mg/kg (64.6±10.3 min.) was observed. The difference between groups was also significant ($p < 0.05$). Increase in duration of sleep is dose dependent (Fig. 4.2).

The leaf extract also did not show any significant difference in onset of sleep between control and treated groups at all doses tested. No significant difference also exists between the groups. However, significant difference ($p < 0.001$) in the duration of sleep was observed between control and extract at 100 and 200 mg/kg (100.4±10.1 and 102.8±15.1min.) and ($p< 0.05$) at 400 mg/kg (75.6±8.4 min.) -Fig. 4.3. The increase in onset and duration of sleep was not dose dependent.

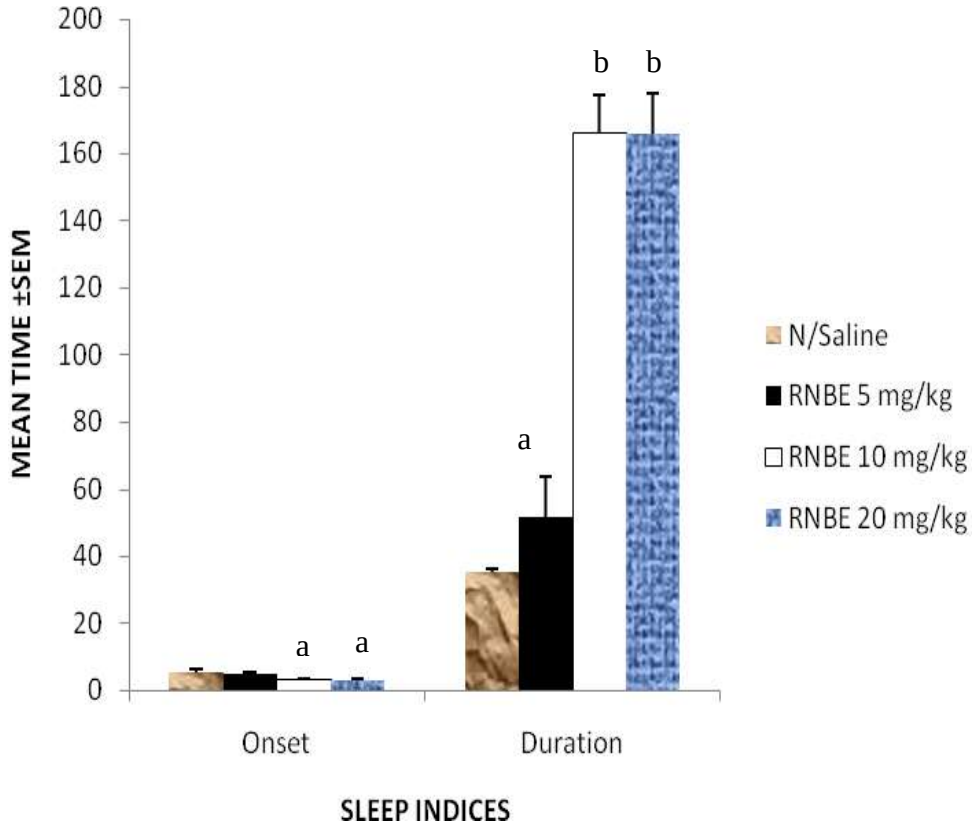


Fig. 4.1: Effect of Stem Bark extract of *Randia nilotica* (RNBE) on onset and duration in Diazepam induced sleep in mice. Significant difference exists between control and treated groups. Key: p<0.05(a); p<0.001(b). One way ANOVA for multiple measures followed by Dunnet's and Scheffe's post hoc tests. N= 5 in each group.

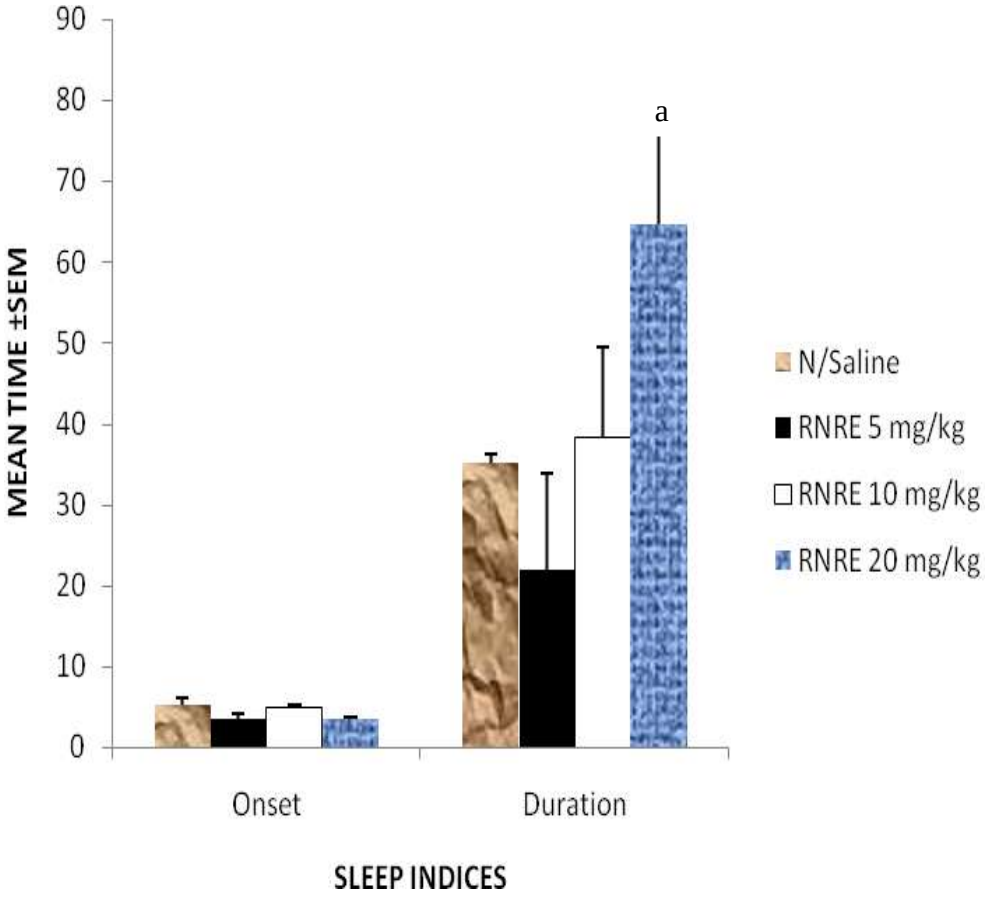


Fig. 4.2 : Effect of Root bark extract of *Randia nilotica* on onset and duration in Diazepam induced sleep in mice. Significant difference between control and treated groups:p<0.05(a) –one way ANOVA for repeated measures followed by Dunnet's and Scheffe's post hoc tests ; N=5 in each group.

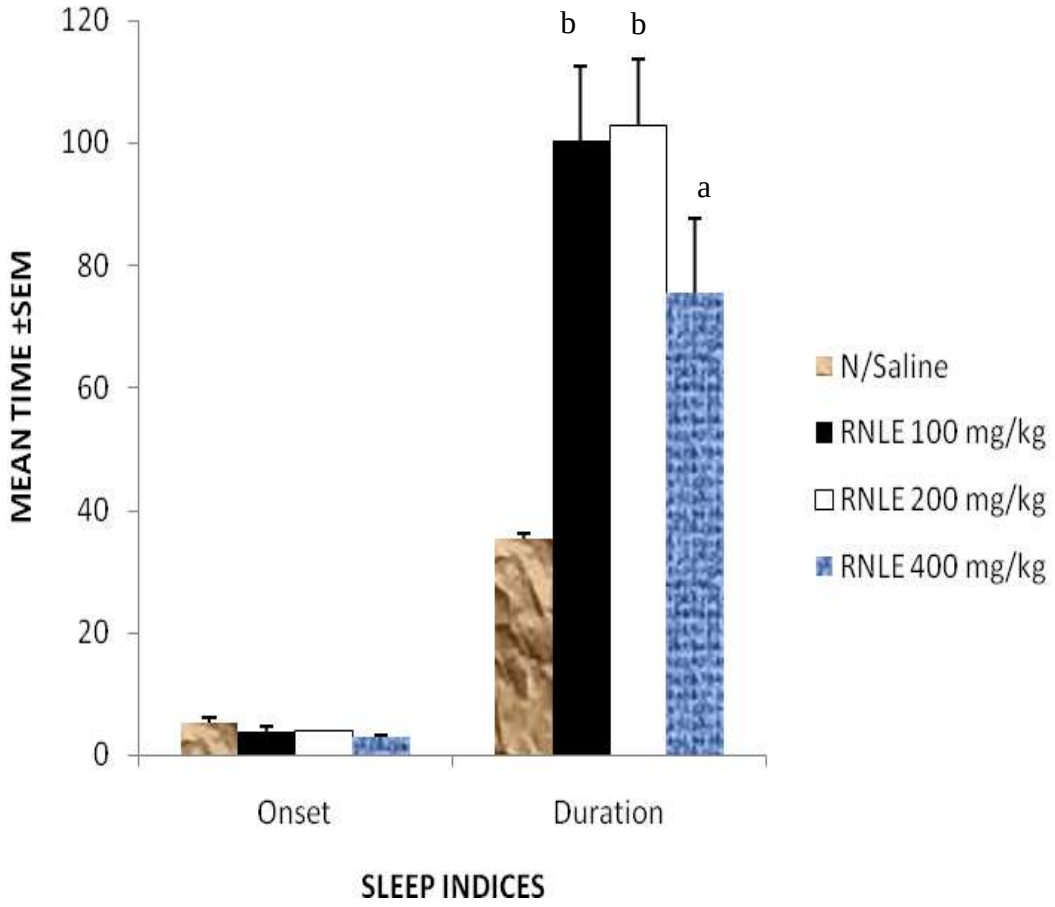


Fig. 4.3: Effect of leaf extract of *Randia nilotica* (RNLE) on onset and duration in diazepam induced sleep in mice. Significant difference between control and treated groups: p<0.05(a) and p<0.001(b) –one way ANOVA followed by Dunnet's and Scheffe's post hoc tests for multiple comparisn; N=5 in each group.

Comparing the three extracts, lowest decreases in onset and highest increases in sleeping time were obtained with the stem bark (20 mg/kg) followed by the leaf extract (200 mg/kg) and then root bark extract (20 mg/kg) - Fig. 4.4

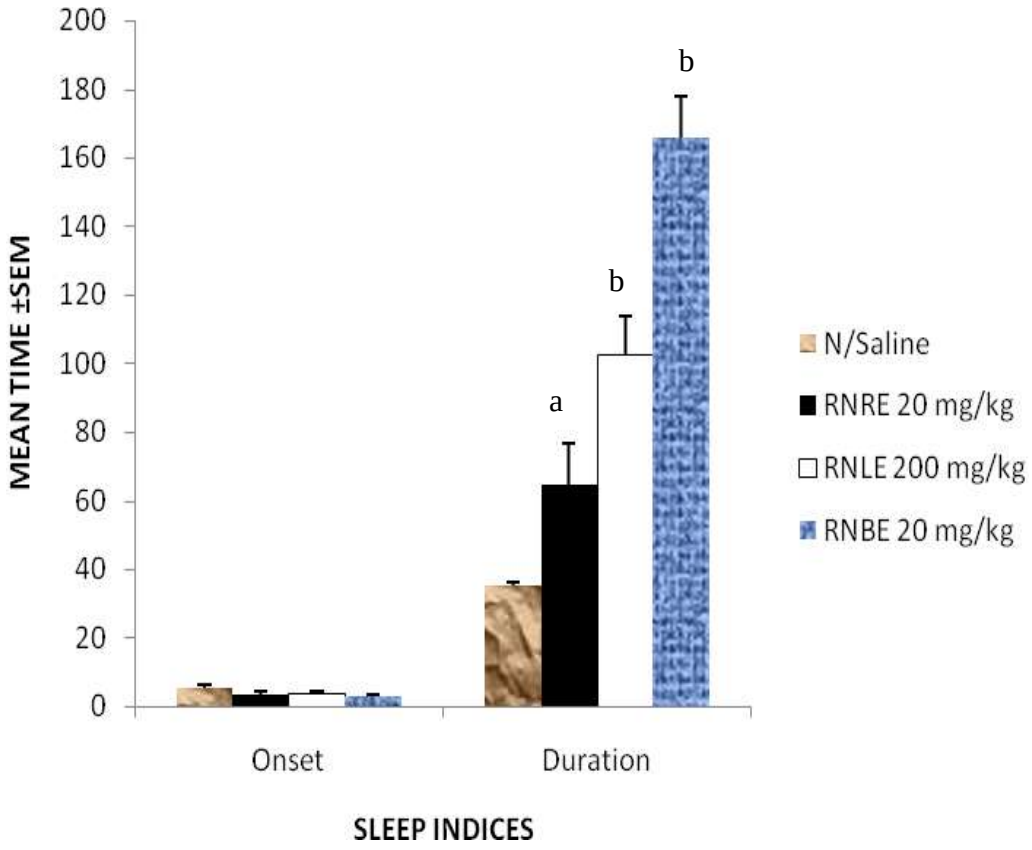


Fig. 4.4: Comparative effects of extracts of the root bark (RNRE), stem bark (RNBE) and leaves (RNLE) of *Randia nilotica* on onset and duration in diazepam induced sleep in mice. Significant difference between control and treated groups: p<0.05(a) and p<0.001(b) –one way ANOVA for multiple measures followed by Dunnet's and Scheffe's post hoc tests. N=5 in each group.

4.2.3 Effect of fractions of RNBE on diazepam induced sleep in mice

Fractions obtained from the stem bark were also subjected to diazepam-induced sleep in mice to determine the most active fraction.

The result of saponin fraction showed significant difference ($p< 0.05$) in onset of sleep between normal saline (5.2±0.7) and 0.5 mg/kg (3.6±0.4) of the extract.The decrease in onset was not dose dependent. The increase in sleep duration however, was dose dependent and was from 35.2±6.0 (Normal saline) to 43.2±6.2 (0.5mg/kg), 90.2±10.2 (1.0mg/kg) and 117.8±8.5 (2.0mg/kg). There was a significant ($p<0.05$) difference between the extract at 2 mg/kg and control. Significant difference ($p< 0.05$) also exists between the groups (Fig. 4.5).

Decrease in onset was seen to be significant ($p < 0.05$) between aqueous fraction at 80 mg/kg (3.4±0.5) and normal saline (5.2±0.7). The decrease however, is not dose dependent. On the other hand, increase in sleep duration was significant ($p < 0.05$) between control and treated groups at all doses tested. It increased from 35.2±6.0 (Normal saline) to 77.0±10 (20mg/kg), 68.2±7.0 (40mg/kg) and 75.0±4.9 (80mg/kg). Increase in sleep time was not dose dependent. Significant difference ($p<0.05$) exist between the groups (Fig. 4.6).

Flavonoidal fraction exhibited a significant difference ($p<0.05$) in onset with control (5.2±0.7) at 20 and 80 mg/kg body weight (3.6±0.2 in each case). The decrease in onset of sleep was not dose dependent. The duration of sleep was observed to decrease with increase in dose which was significantly different with control ($p<0.05$) at 20 mg/kg body weight. It decreased from 68.6±11.0 (20mg/kg) to 60.6±10.0 (40 mg/kg) and 55.2±3.7 (80 mg/kg). No significant difference existed between groups (Fig. 4.7)

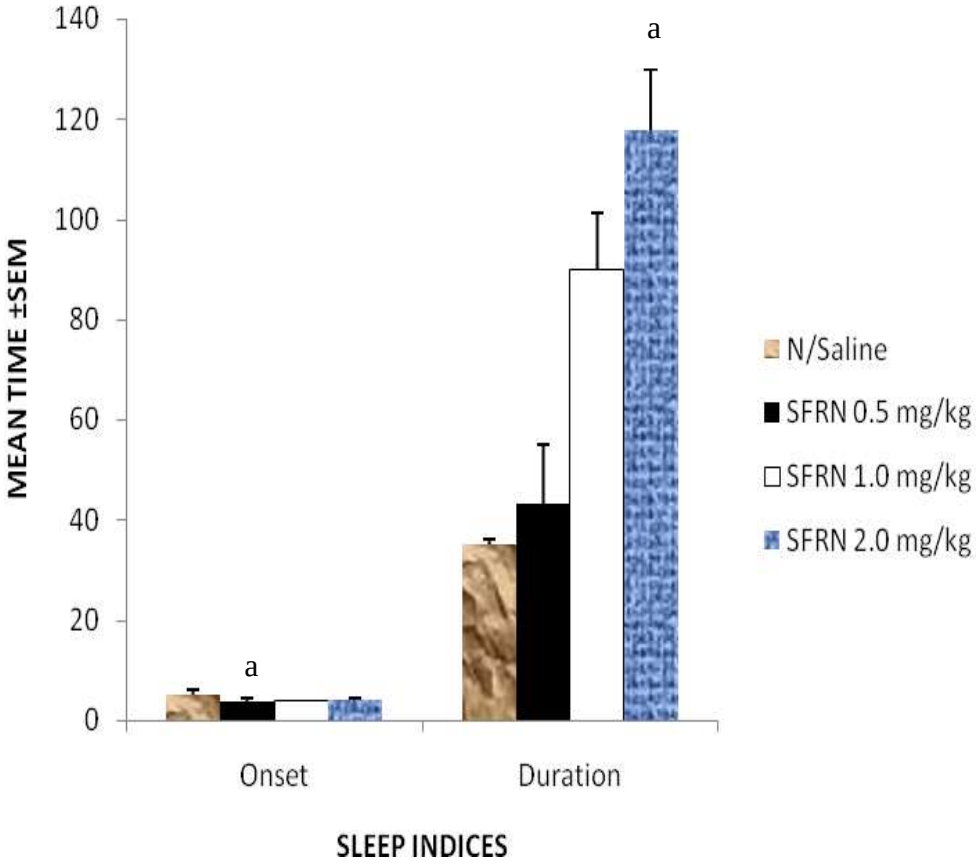


Fig. 4.5: Effect of saponin fraction of *Randia nilotica* (SFRN) stem bark extract on onset and duration in diazepam induced sleep in mice. Significant difference between control and treated groups: p<0.05(a) –one way ANOVA for multiple measures followed by Dunnet's and Scheffe's post hoc tests.N=5 in each group.

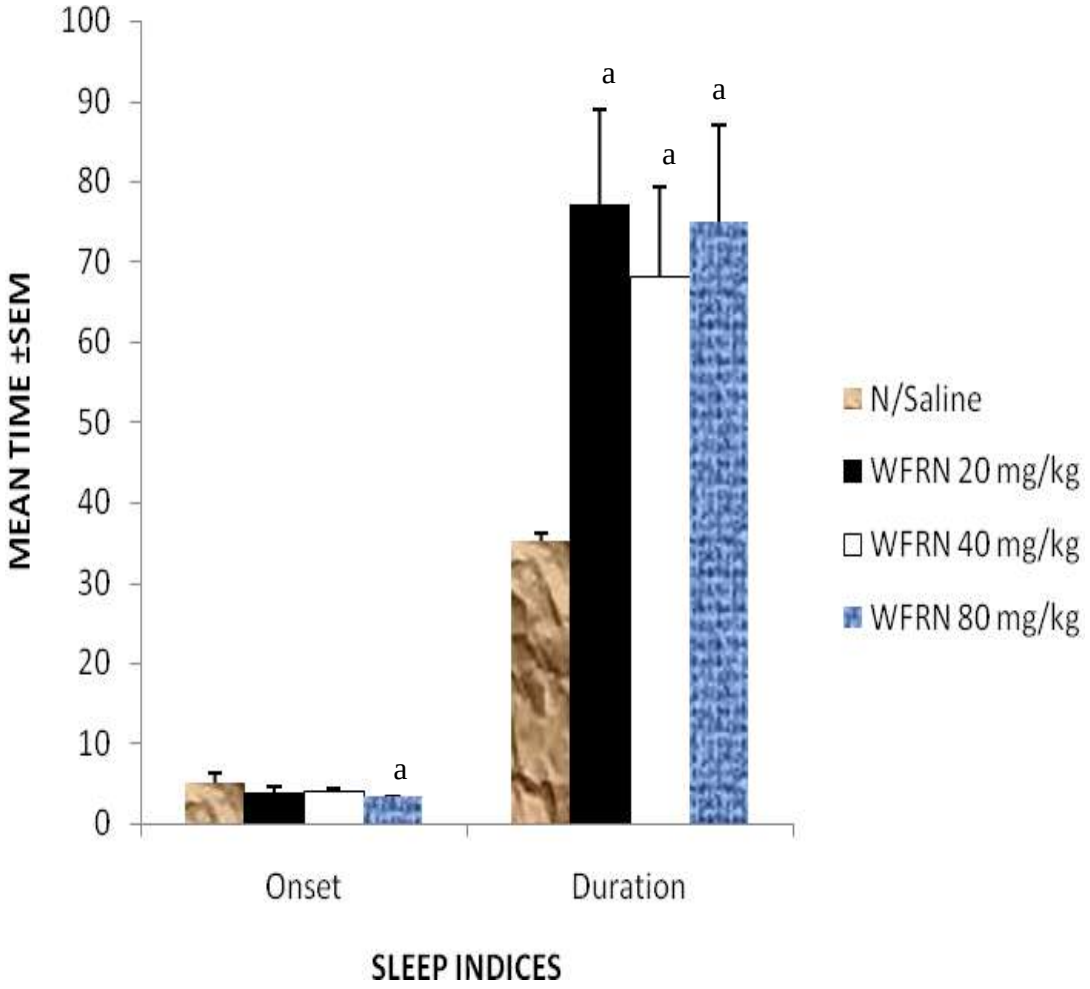


Fig. 4.6: Effect of aqueous fraction of *Randia nilotica* (WFRN) on onset and duration in the diazepam induced sleep in mice. Significant difference between control and treated groups: p<0.05(a) –one way ANOVA for multiple measures followed by Dunnet's and Scheffe's post hoc tests.N=5 in each group.

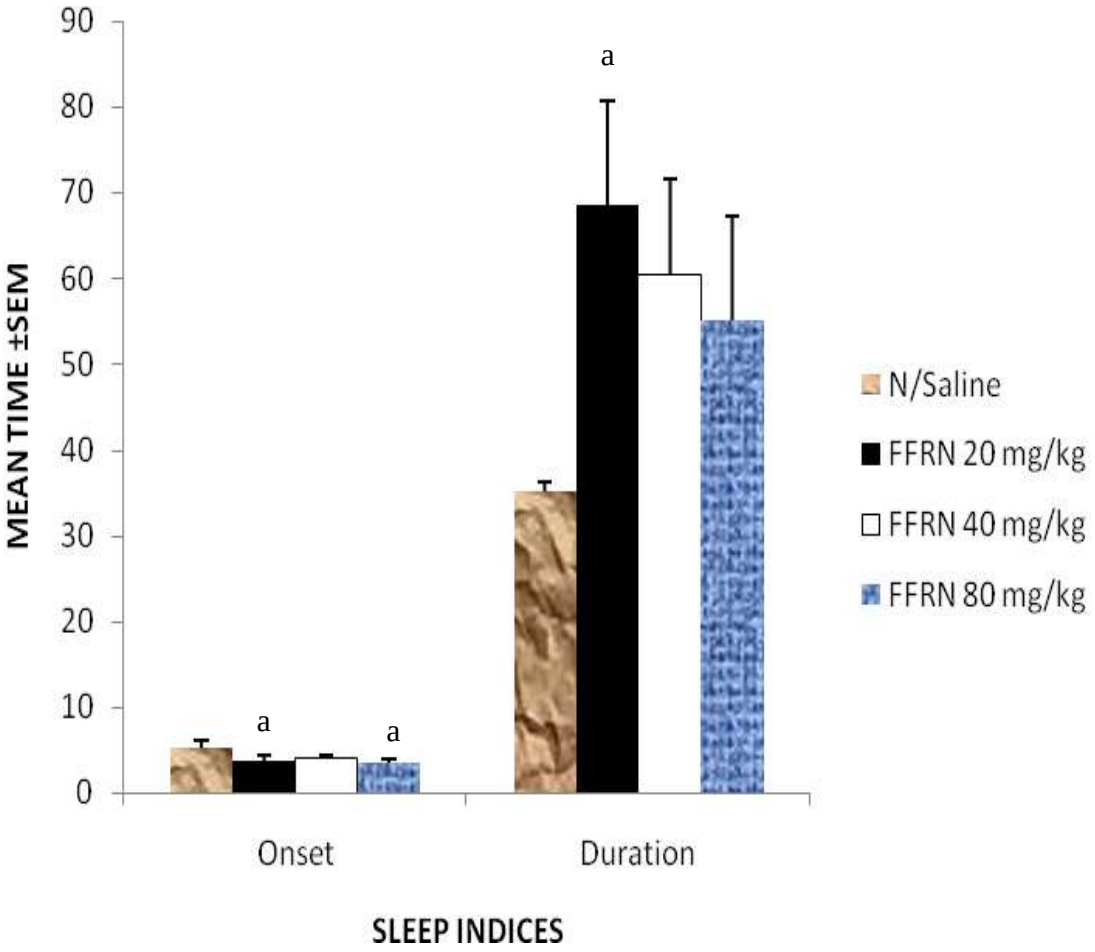


Fig. 4.7: Effect of flavonoid fraction of *Randia nilotica* (FFRN) stem bark extract on onset and duration sleep in diazepam induced sleep in mice. Significant difference between control and treated groups: p<0.05(a) –one factor ANOVA for multiple measures followed by Dunnet's and Scheffe's post hoc tests.N=5 in each group.

Comparative sleep effects produced by various fractions showed high potency for the saponins. Lowest decrease in onset and highest increase in sleeping time was observed with this fraction (Fig. 4.8).

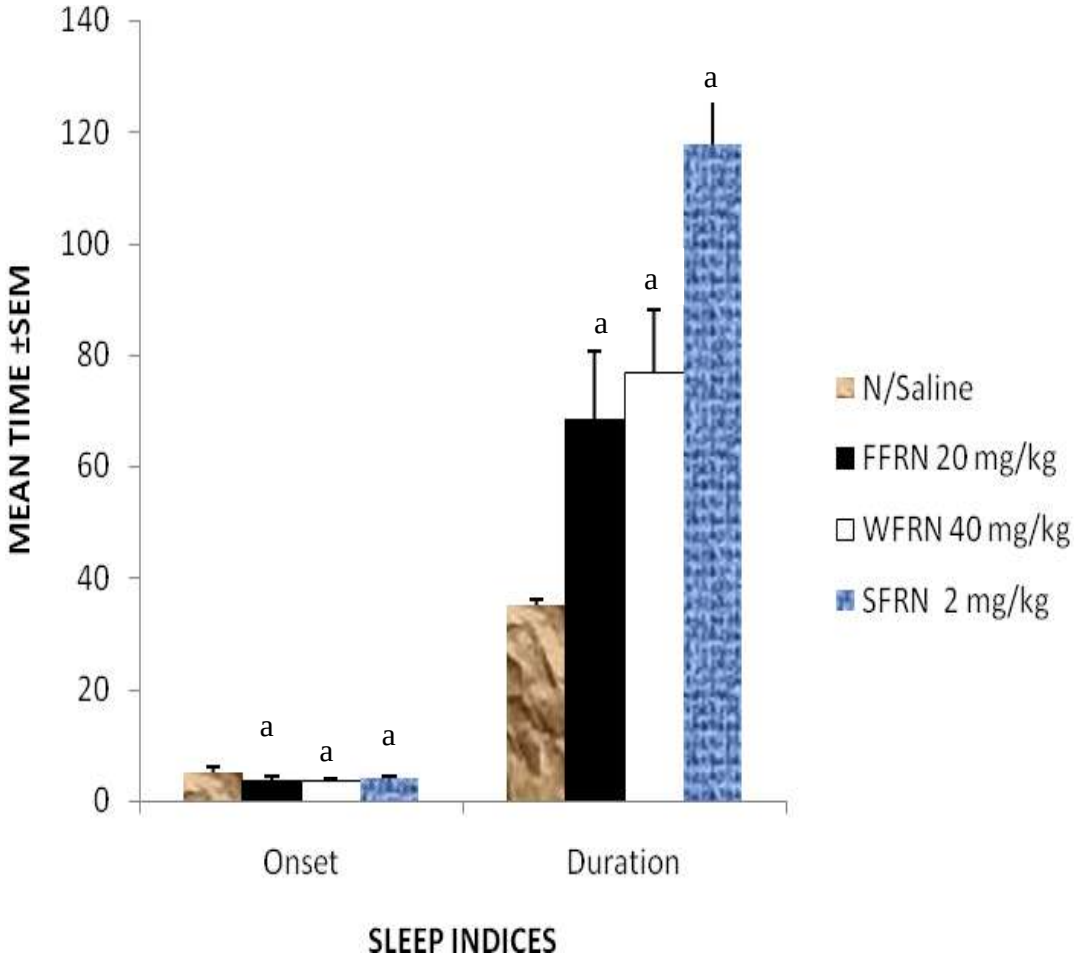


Fig.4.8: Comparative effects in sleeping time of the saponin (SFRN), aqueous (WFRN) and flavonoid (FFRN) fractions of *Randia nilotica* stem bark extract on onset and duration in diazepam induced sleep in mice. Significant difference between control and treated groups: p<0.05(a) -one factor ANOVA followed by Dunnet's and Scheffe's post hoc tests.N=5 in each group.

4.2.4 Effect of saponin sub fractions of RNBE on diazepam induced sleep in mice

The subfractions obtained from column chromatography were also tested using diazepam induced sleep in mice.

Result of the SF_1 subfraction showed a dose dependent decrease in onset of sleep which was not significant compared with control. Increase in duration of sleep was also dose dependent. The increase is significant ($p<0.001$) at 0.5 mg/kg (121.3±11.7 min.) compared with normal saline (19.0±3.7). Significant difference ($p<0.001$) also exists between the groups (Fig. 4.9)

SF_2 subfraction showed no significant difference in sleep onset between control and treated groups. The decrease was also not dose dependent. Increase in sleep duration was however, dose dependent. There is a significant difference ($p<0.05$) between normal saline (19.0±3.7) and treated groups - 38.0±6.3min.(0.125mg/kg), 77.3±6.5min.(0.25mg/kg) and 82.8±8.6 min.(0.5 mg/kg) -Fig. 4.10.

The SF_3 sub-fraction also showed a dose dependent decrease in onset of sleep which is significant ($p<0.05$) at 0.5 mg/kg body weight compared to control. Increase in duration of sleep was however observed to be not dose dependent, but significantly ($p<0.05$) different at 0.5 mg/kg compared with control (Fig 4.11).

The SF_4 subfraction did not show any significant decrease in onset compared with control. No significant difference among groups was observed as well. The extract however, showed significant difference in duration of sleep ($p<0.05$) with control at all doses tested. Significant difference ($p<0.05$) also exists among the groups (Fig. 4.12).

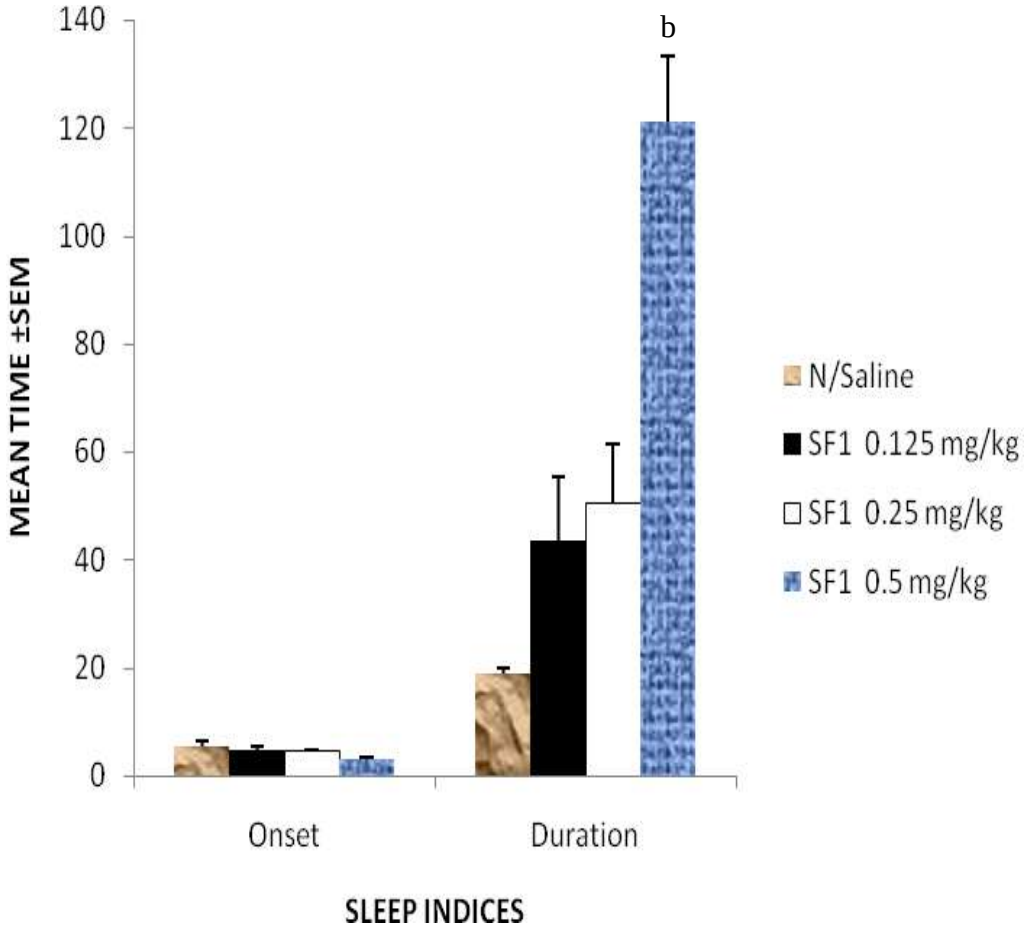


Fig. 4.9: Effect of the first saponin sub fraction (SF_1) on onset and duration of diazepam induced sleep in mice. Significant difference between control and treated groups: p<0.001(b)-one factor ANOVA for multiple measures followed by Dunnet's and Scheffe's post hoc tests. N=5 in each group.

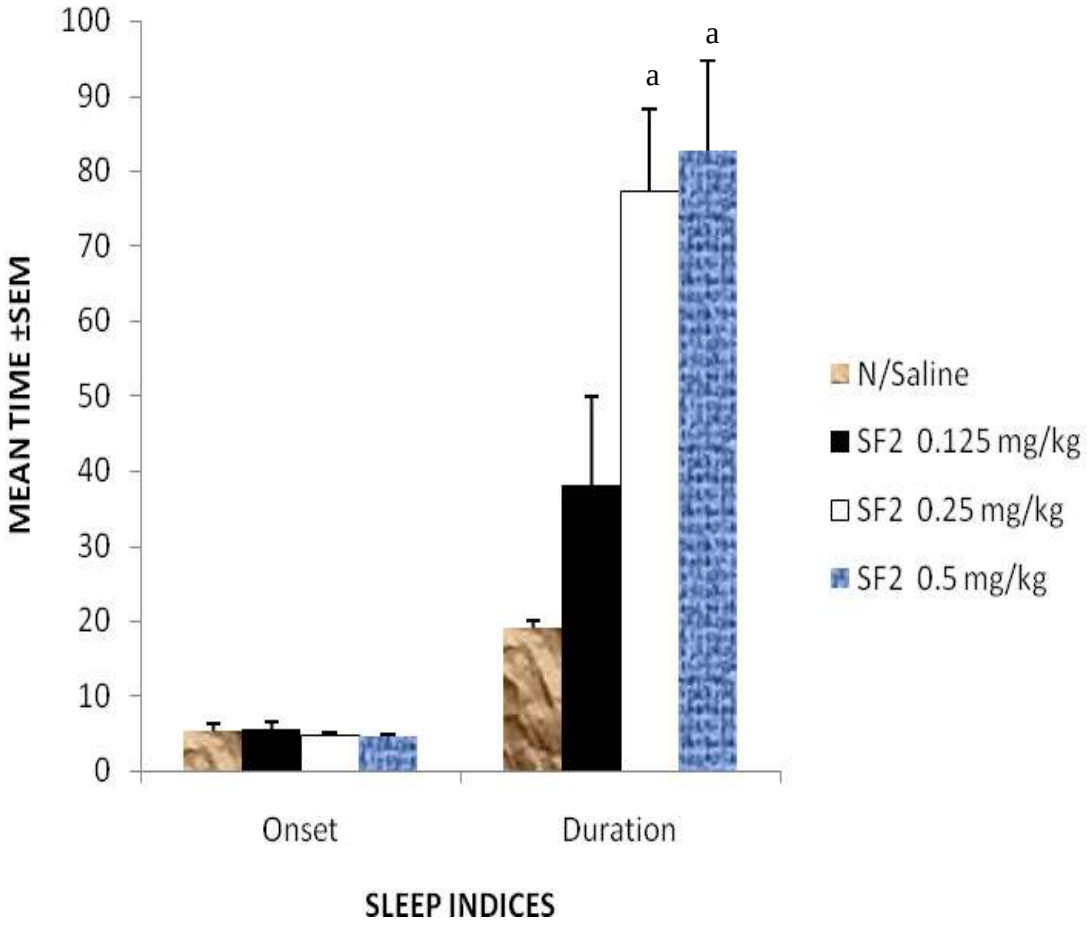


Fig. 4.10: Effect of second saponin sub fraction (SF_2) on onset and duration in diazepam induced sleep in mice. Significant difference between control and treated groups: p<0.05(a)-one factor ANOVA for multiple measure followed by Dunnet's and Scheffe's tests of multiple comparison.N=5 in each group.

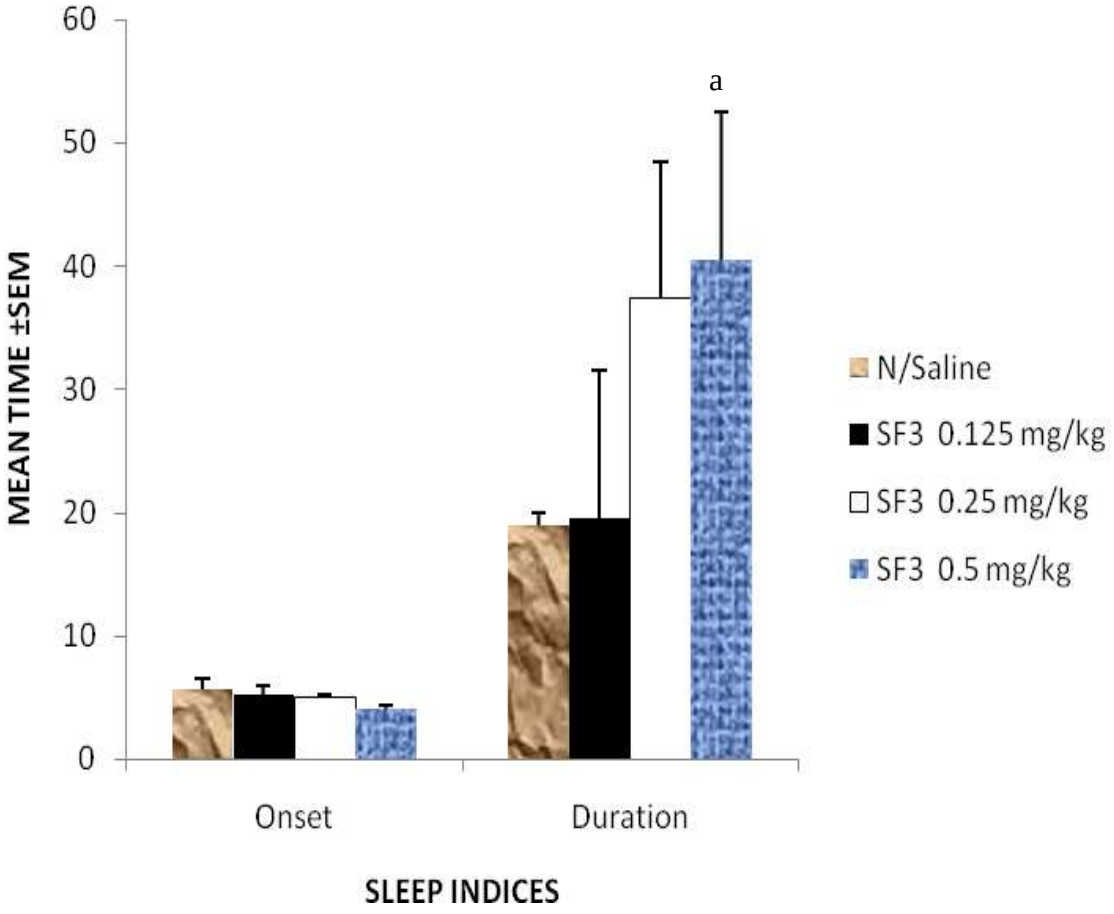


Fig. 4.11: Effect of the third saponin sub fraction (SF_3) on onset and duration in diazepam induced sleep in mice. Significant difference between control and treated groups: p<0.05(a)-one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparison.N=5 in each group.

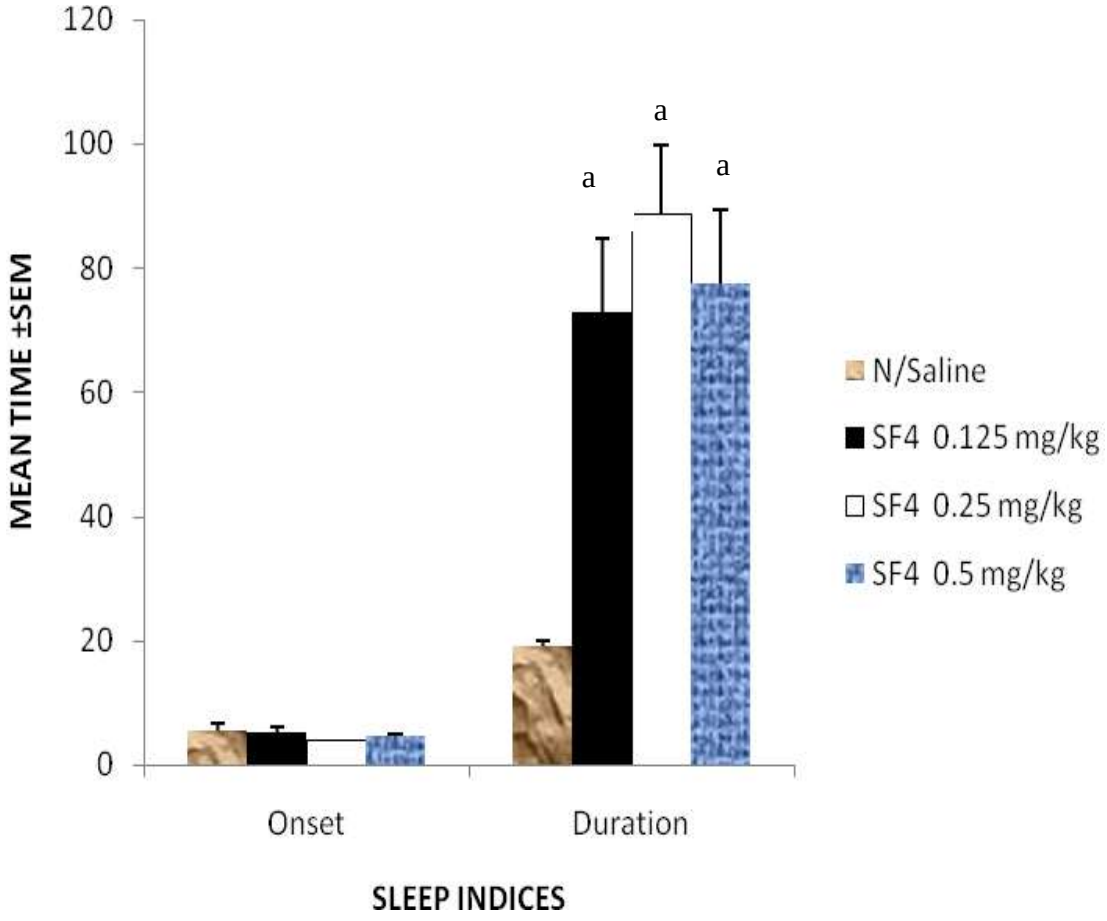


Fig. 4.12: Effect of fourth saponin sub fraction (SF_4) on onset and duration in diazepam induced sleep in mice. Significant difference between control and treated groups: p<0.05(a)-one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparison.N=5 in each group.

Result of sleeping time obtained from various sub fractions when compared, showed that the SF_1 produced the least decrease in onset and highest increase in duration of sleep followed by SF_4 and then SF_2 (Fig. 4.13).

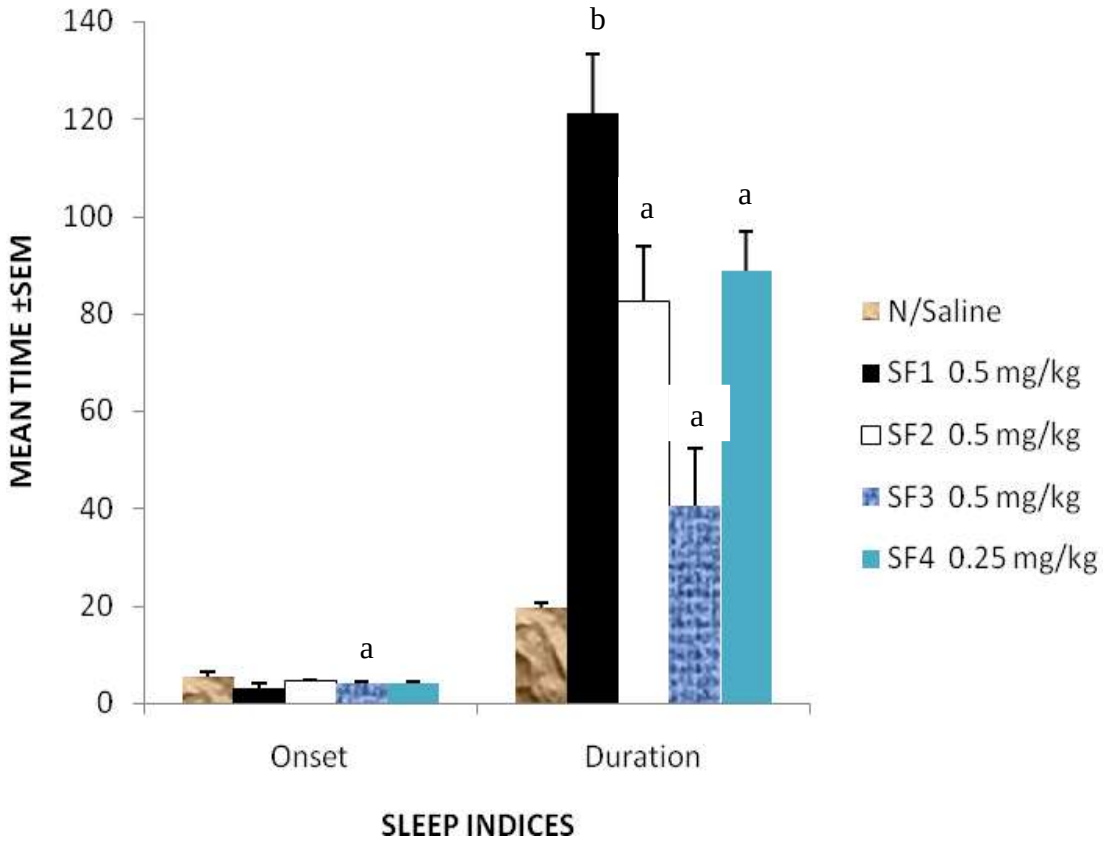


Fig. 4.13: Comparative sedative effects of sub fractions of saponin from *Randia nilotica* stem bark extract on onset and duration in diazepam induced sleep in mice. Significant difference between control and treated groups: $p<0.05$(a) and $p<0.001$(b)-one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparison.

4.3 Other Behavioural Studies

4.3.1 Effect of extracts of *Randia nilotica* on Exploratory Activity in Mice

The HA stem bark extracts (5, 10 and 20 mg/kg body weight i.p) exhibited a dose-dependent decrease in the number of head dips in the hole-board experiments. Mean number of head dips decreased from 7.66±2.5 (normal saline) to 6.2±1.8 (5 mg/kg), 4.5±1.5 (10 mg/kg), 1.3±0.8 (20 mg/kg) and 2.2±0.8 (Diazepam, 1 mg/kg). The decrease was significant at 20 mg/kg ($p < 0.05$). Diazepam also caused a significant decrease in exploratory activity (Fig. 4.14).

Similarly, the saponin fraction exhibited a significant ($P<0.05$) dose-dependent decrease in the number of head dips in this experiment at 0.5 mg/kg (2.0±0.6 head dips), 1.0 mg/kg (1.2±0.4) body weight and ($p<0.005$) at 2.0 mg/kg (0.3±0.0) compared to normal saline (7.66±2.5) and diazepam (2.2±0.8) controls (Fig. 4.15).

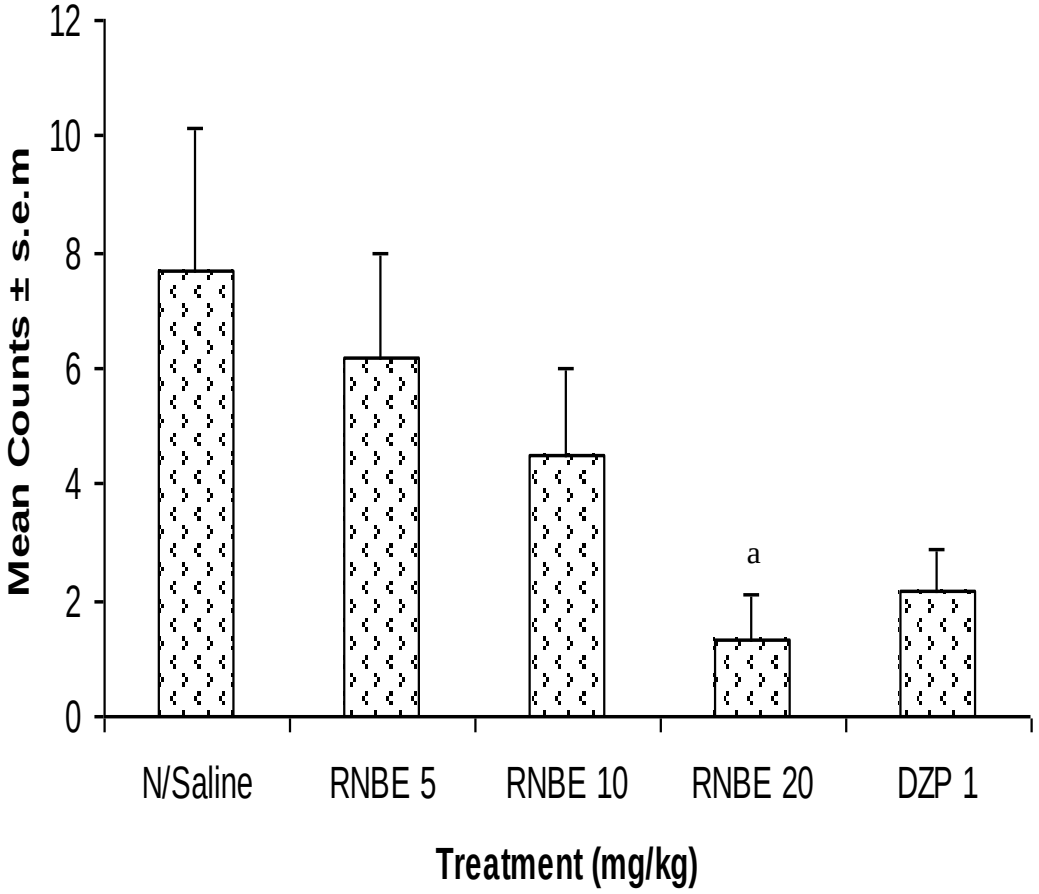


Fig. 4.14: Effect of stem bark extract of *Randia nilotica* on exploratory behaviour in mice. Significant difference between control and treated groups: p<0.05(a)-one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparison. N=5 in each group.

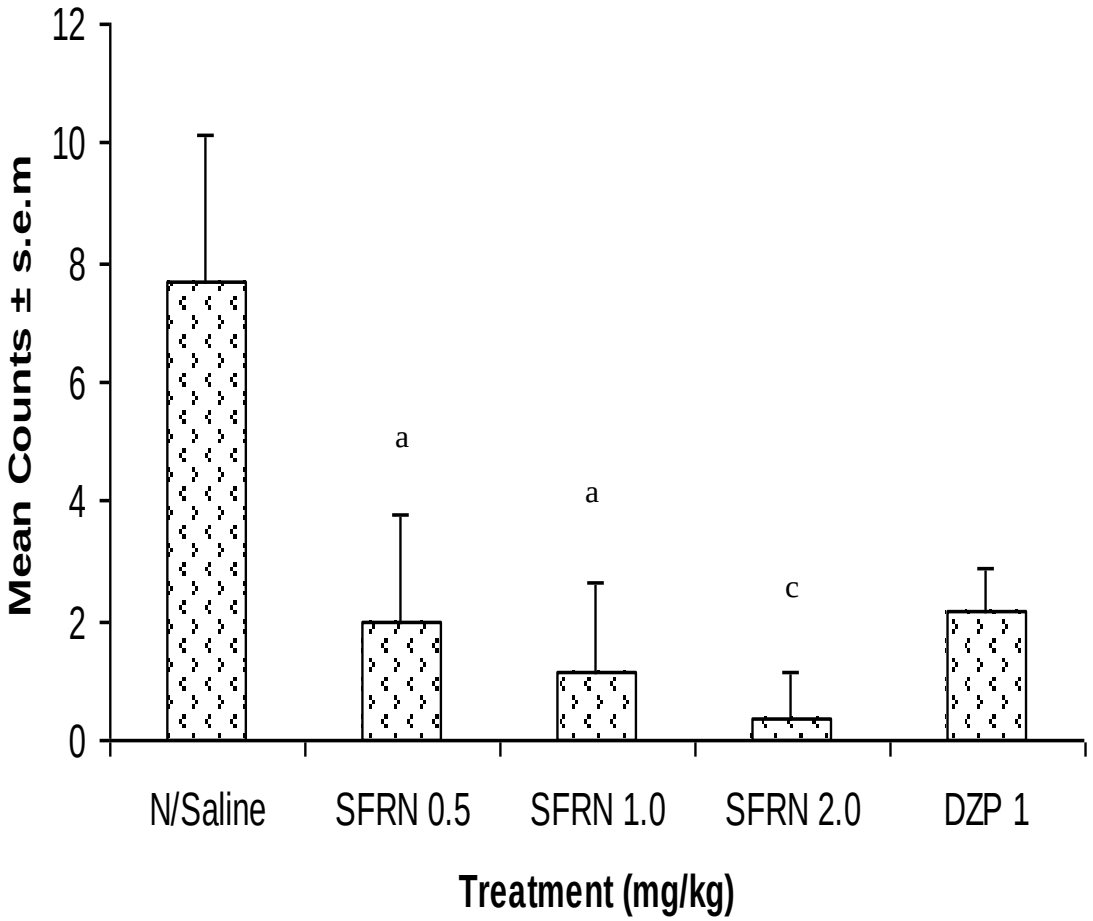


Fig. 4.15: Effect of saponin fraction of the stem bark extract of *Randia nilotica* (SFRN) on exploratory behaviour in mice. Significant difference between control and treated groups: p<0.05(a) and p<0.005(c) –one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparison. N=5 in each group.

4.3.2 Effect of extracts of *R. nilotica* on Pentobarbitone – Induced Sleep in Mice

The HA stembark extract of *R. nilotica* decreased onset of sleep which was significant ($p<0.001$) at 20 mg/kg body weight from 12.3±1.1 to 3.4±0.2 minutes. Significant difference between groups was also observed ($p<0.001$). The extract also significantly prolonged the duration of pentobarbitone-induced sleep ($p<0.01$) at 5 mg/kg (39±6.3 min.), 10 mg/kg (37±6.6 min.) and ($p<0.001$) at 20 mg/kg (162.2±10.0 min.) compared to control (6.1±1.3 min.). Diazepam (1 mg/kg) also significantly ($p<0.001$) increased duration and reduced onset of sleep from 6.1±1.3 to 71.7±8.6 minutes and from 12.3±1.1 to 3.5±0.2 minutes respectively (Fig. 4.16).

Similarly, saponin fraction of *R. nilotica* produced a dose dependent decrease in onset of sleep and a dose-dependent prolongation of pentobarbitone-induced sleep. These were however not significant compared with control (Fig. 4.17).

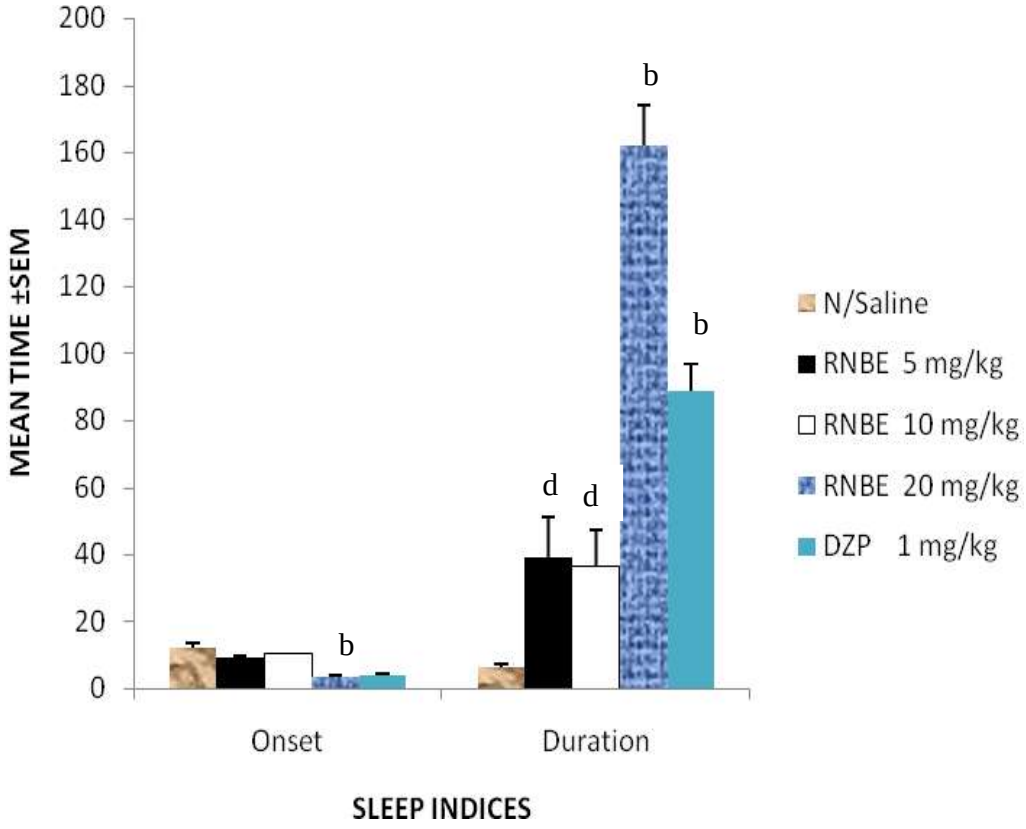


Fig. 4.16: Effect of stem bark extract of *Randia nilotica* (RNBE) and diazepam (DZP) on onset and duration in pentobarbitone induced sleep in mice. Significant difference between control and treated groups: p≤ 0.01(d) and p<0.001(b) –one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparison. N= 5 in each group.

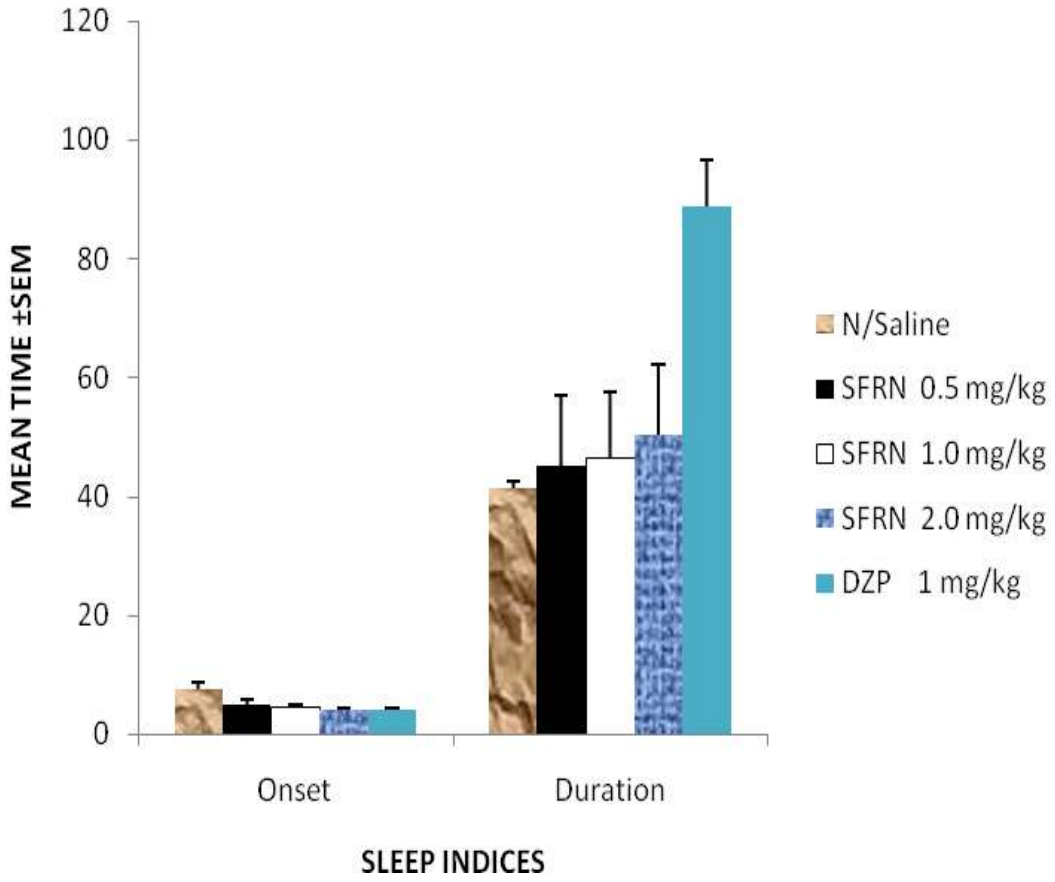


Fig. 4.17: Effect of saponin fraction of *Randia nilotica* (SFRN) on onset and duration in the pentobarbitone induced sleep in mice. No significant difference between control and treated groups (one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparison). N=5 in each group.

4.3.3 Effect of extracts of *Randia nilotica* on Motor Co-ordination in mice

The HA stem bark extract (5, 10 and 20 mg/kg body weight i.p) did not produce any significant observable effect on motor co-ordination as determined by the treadmill device (Table 4.6).

Similarly, the saponin fraction of the plant extract did not produce any observable effect on motor co-ordination (Table 4.7)

Table 4.6: Effect of stem bark extract of *Randia nilotica* (RNBE) on motor coordination in mice.

	TIME AFTER TREATMENT(MINUTES)							
TREATMENT		30		60		90		120
	Time on rod	Fail. %	Time on rod	Fail. %	Time on rod	Fail. %	Time on rod	Fail. %
CONTROL	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0
RNBE 5mg/kg	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0
RNBE 10mg/kg	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0
RNBE 20mg/kg	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0

Results are presented as mean ± SEM, N=6 in each group.

Table 4.7: Effect of saponin fraction of *Randia nilotica* (SFRN) on motor coordination in mice.

TREATMENT	TIME AFTER TREATMENT (MINUTES)							
		30		60		90		120
	Time on rod	Fail. %	Time on rod	Fail. %	Time on rod	Fail. %	Time on rod	Fail. %
CONTROL	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0
SFRN .5mg/kg	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0
SFRN 1.0mg/kg	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0
SFRN 2.0mg/kg	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0	>180.0±0.0	0.0

Results are presented as mean ± SEM, N=6 in each group.

4.3.4 Effect of extracts of *Randia nilotica* on the Beam Walk Assay in Mice

The crude hydroalcoholic stem bark extract did not show any significant difference with control in time to reach goal box at all doses tested (Fig. 4.18). Diazepam 1.5 mg/kg did not produce any difference either. The mean number of foot slips was also not significantly different from control. Diazepam however, exhibited a significant ($p<0.001$) difference with control and with extracts ($p<0.05$) at all doses tested (Fig. 4.19).

Similarly, the saponin fraction had no effect on time to reach goal box as there was no significant difference between it and the control (Fig. 4.20). It also had little effect on number of foot slips compared with the control. Diazepam however, showed a significant ($p<0.001$) increase in number of foot slips compared with the control and with the extracts ($p<0.05$) at the doses tested (Fig. 4.21).

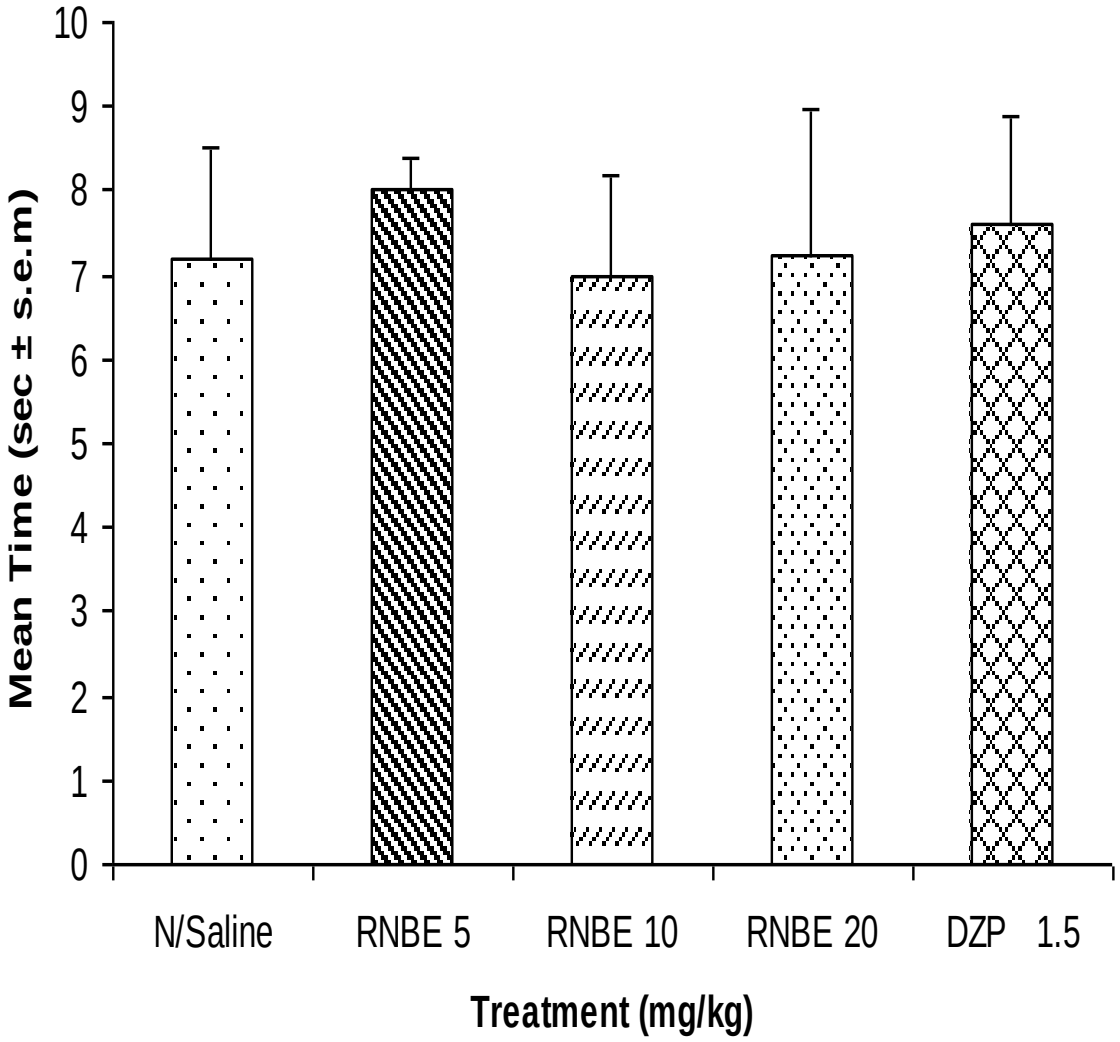


Fig. 4.18: Effect of stem bark extract of *Randia nilotica* (RNBE) on time to reach goal box in the beam walking assay in mice. No significant difference between control and treated groups (one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparison). N= 6 in each group.

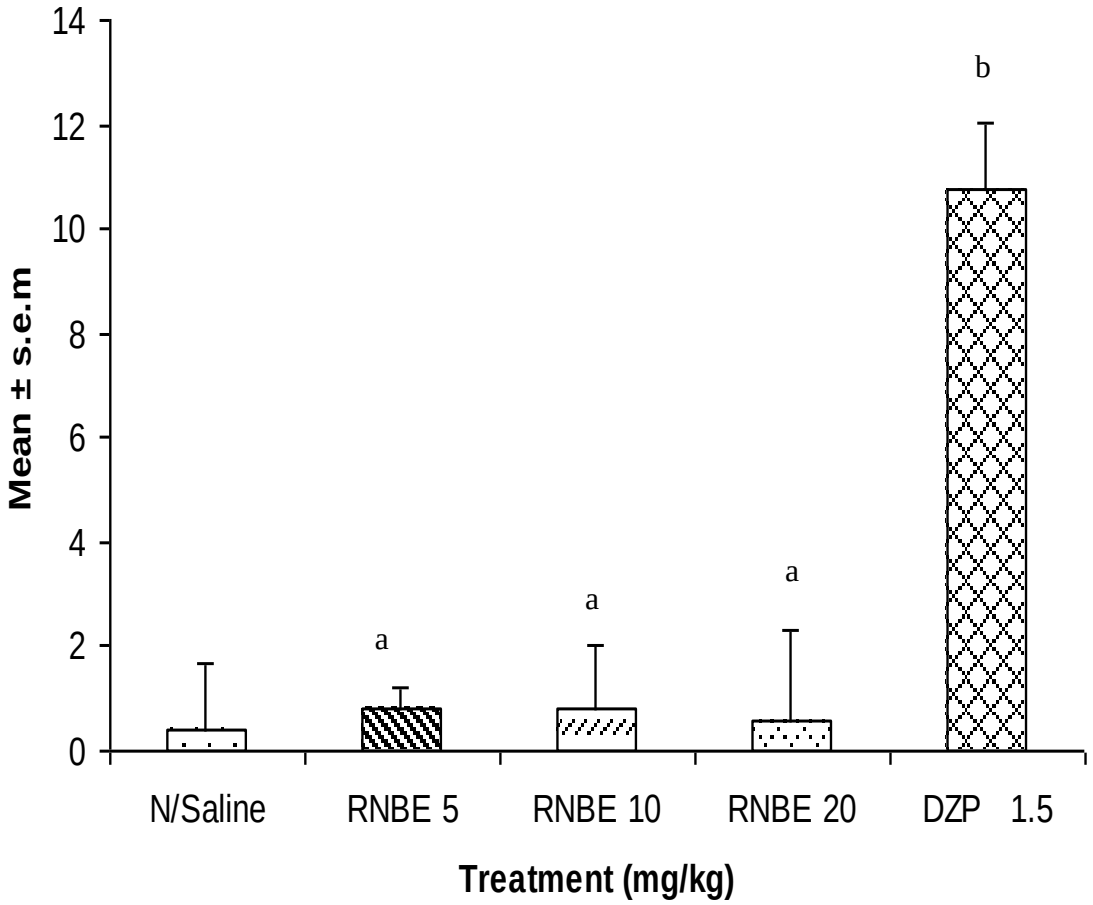


Fig. 4.19: Effect of stem bark extract of *Randia nilotica* (RNBE) and diazepam on foot slips in the beam walking assay in mice. Significant difference between control and diazepam (p±0.001 -b) and between diazepam and extracts (p±0.05 -a) –one way ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests. N=6 in each group.

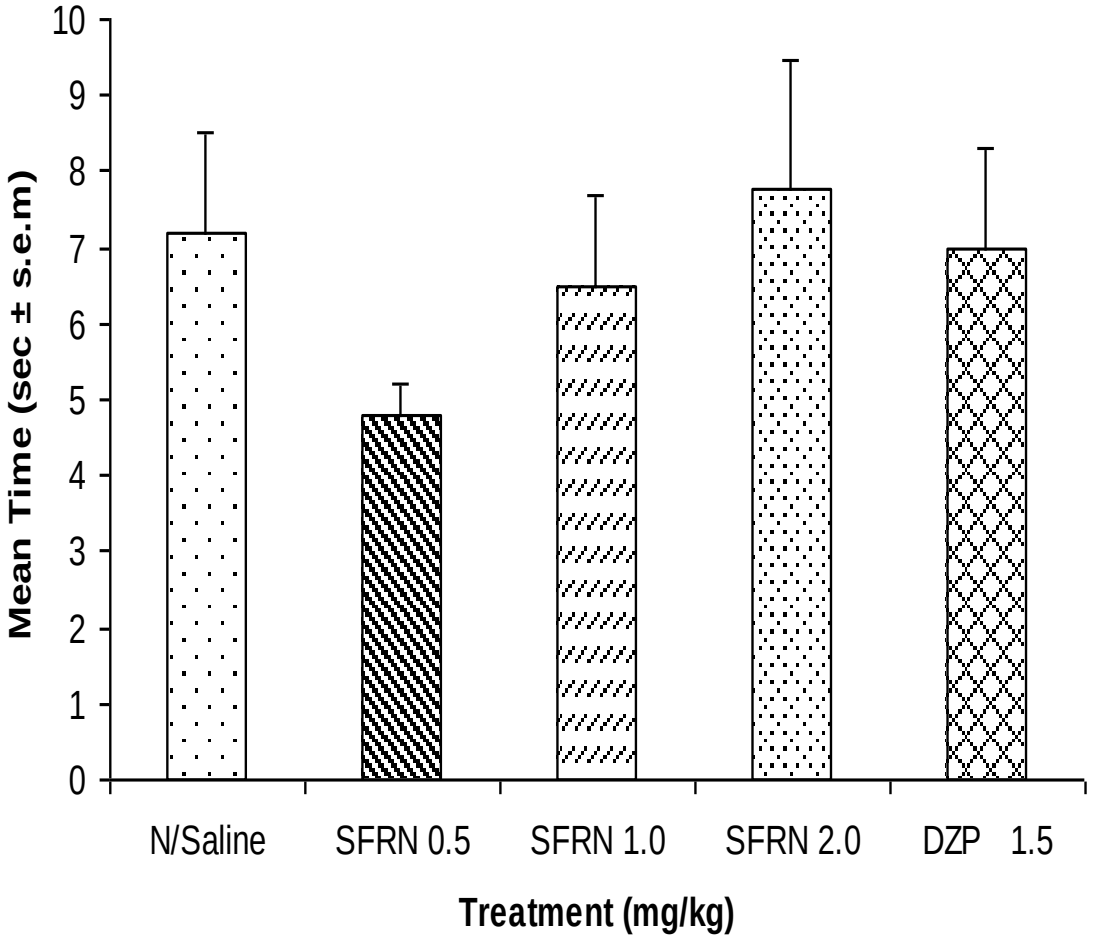


Fig. 4.20: Effect of saponin fraction of *Randia nilotica* (SFRN) on time to reach goal box in the beam walking assay in mice. No significant difference between control and treated groups (one way ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparisn). N=6 in each group.

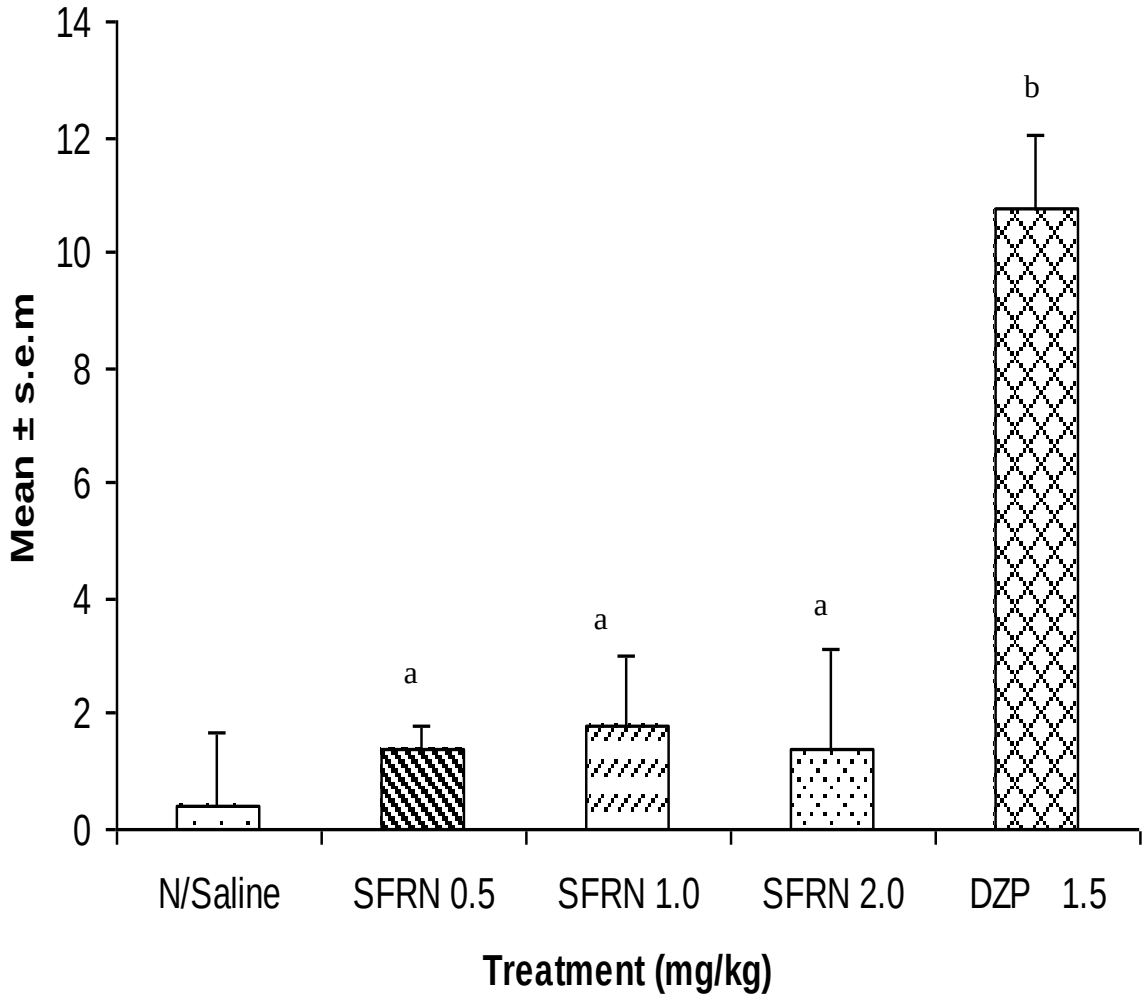


Fig. 4.21: Saponin fraction of *Randia nilotica* (SFRN), diazepam (DZP) and number of foot slips in beam walk assay in mice. Significant difference between control and diazepam (p≤0.001 -b) and between diazepam and extracts (p≤0.05 -a)-one way ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests. N=6 in each group.

4.3.5 Effect of extract of *R. nilotica* on amphetamine – induced stereotype behaviour in mice

The HA stem bark extract (5, 10 and 20 mg/kg body weight i.p) had a biphasic effect on amphetamine-induced stereotyped behaviour in mice (Fig. 4.22). There was a significant reduction in jumping/climbing episodes ($p<0.001$) produced by the extracts and chlorpromazine compared to the control. Significant difference also existed between the groups ($p<0.001$).

Reduction in sniffing was observed with extract at 5 mg/kg body weight which was significant ($p<0.05$) compared with the control. Chlorpromazine 2 mg/kg also produced similar effect. Higher doses paradoxically produced increases rather than decrease in sniffing. The increase was higher with 20 mg/kg dose of the extract.

The extract attenuated mean count in limb licking at 20 mg/kg. Chlorpromazine had a similar effect. At 5 and 10 mg/kg, a significant ($p<0.05$) increase in limb licking was observed compared with control (Fig.4.22).

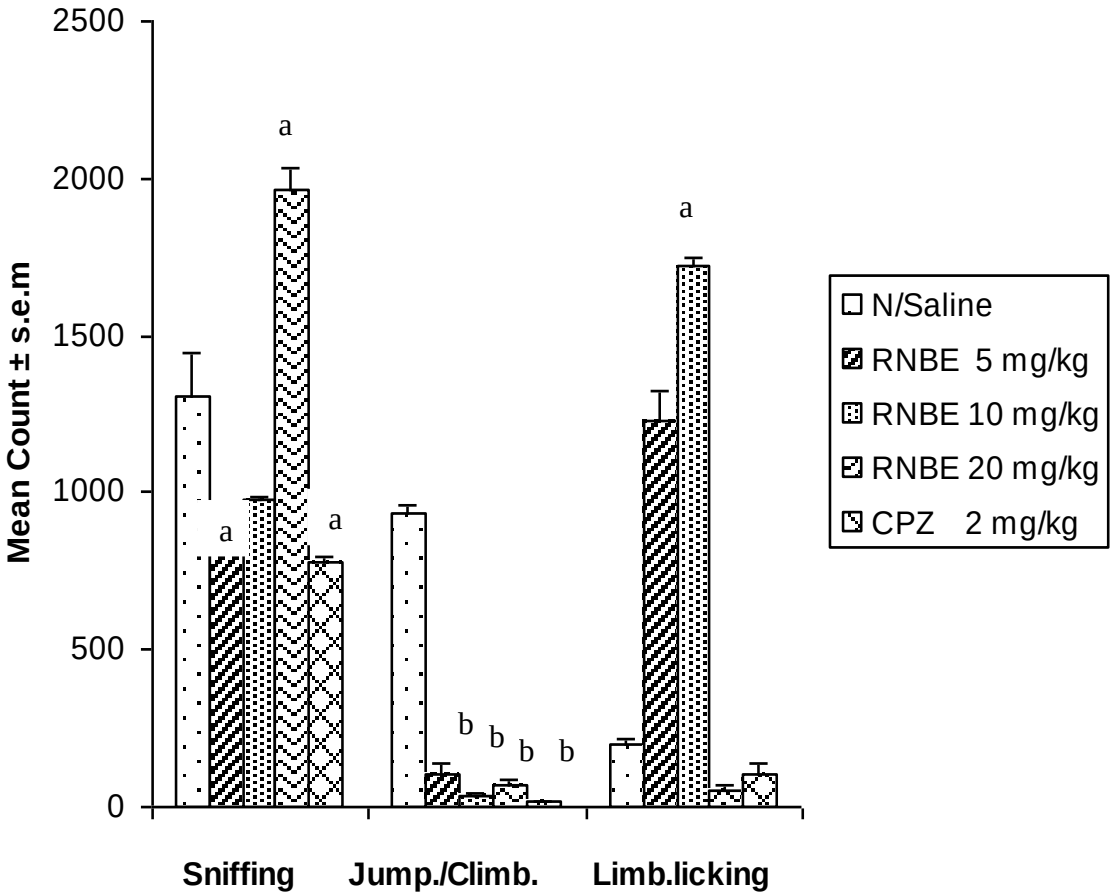


Fig. 4.22: Effect of hydroalcoholic stem bark extract of *Randia nilotica* (RNBE) on amphetamine induced stereotyped behaviour in mice. Significant difference between control and treated groups: p≤ 0.05(a) and p≤ 0.001(b)-one way ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparisn.

4.4 Anticonvulsant Studies

4.4.1 The effect of *R. nilotica* extracts on maximal electroshock test (MEST) in chicks and mice

The crude HA stembark extract (5, 10 and 20 mg/kg body wt.) protected chicks (50%, 50% and 90% respectively) against hind limb tonic extension (HLTE) in the maximal electroshock induced convulsion test. Phenobarbitone-PBT (20 mg/kg body weight i.p) used as positive control had the same effect (90% protection) with the crude extract at 20 mg/kg body weight i.p. (Fig 4.23).

Similarly, the saponin fraction of the stembark of *R. nilotica* protected mice against HLTE in the maximal electroshock-induced convulsion tests (Fig. 4.24).

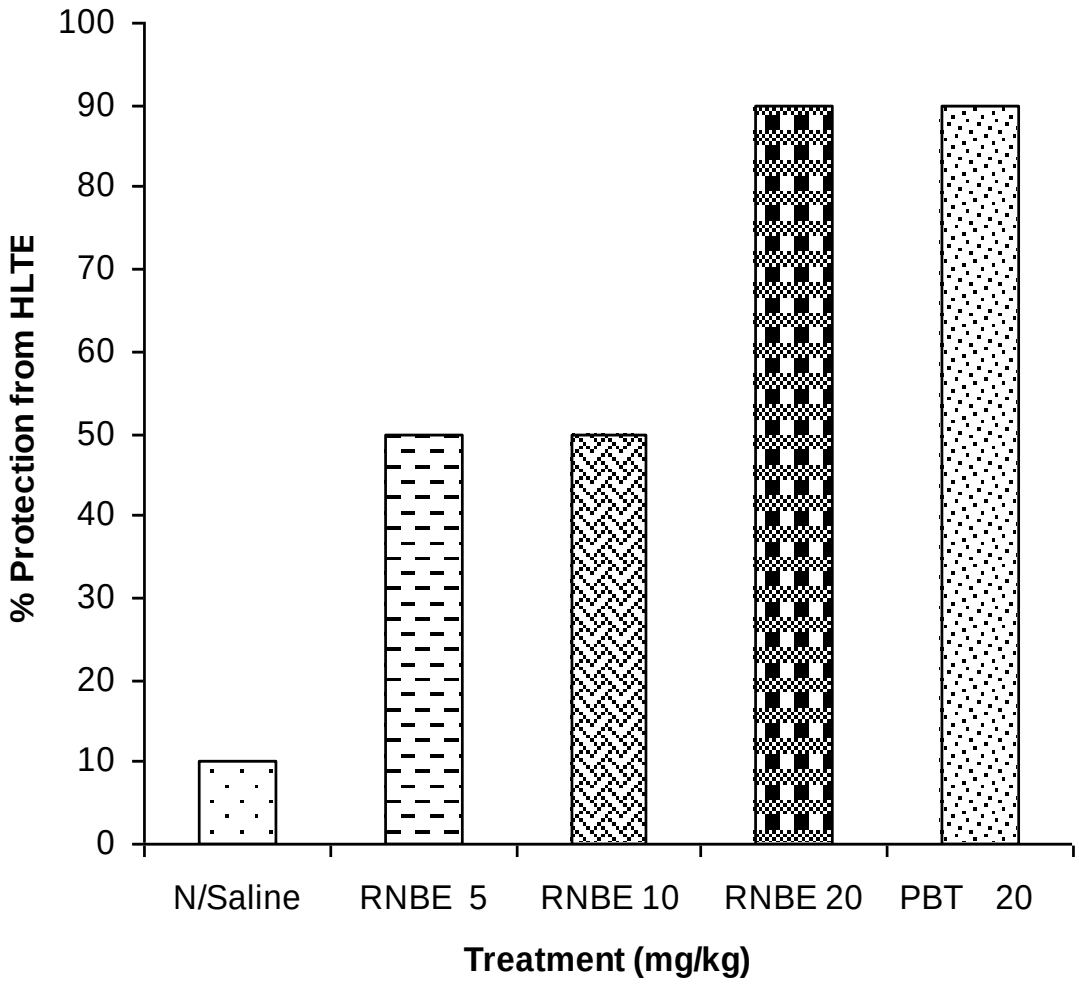


Fig. 4.23: Effect of hydroalcoholic stem bark extract of *randia nilotica* (RNBE) and phenobarbitone (PBT) on HLTE phase in chicks using maximum electroshock test.

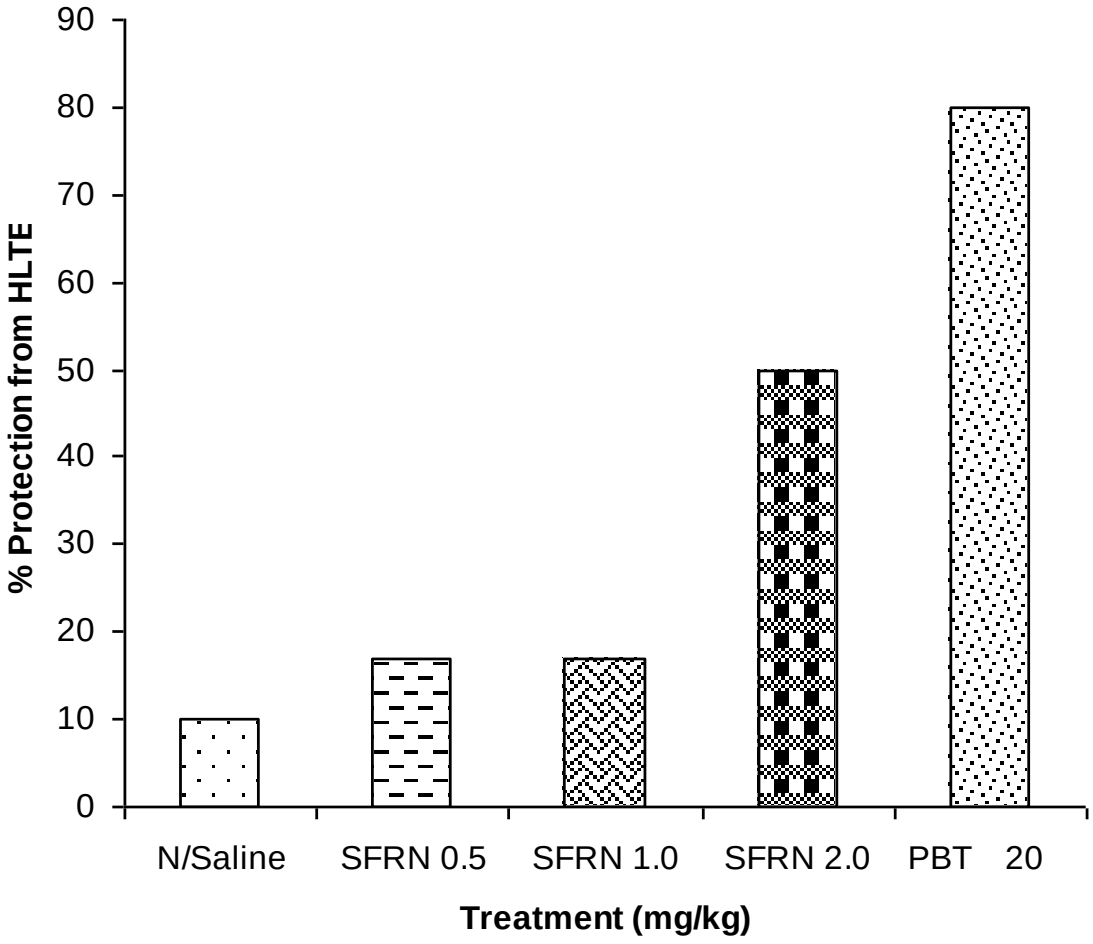


Fig. 4.24: Effect of saponin fraction of *Randia nilotica* (SFRN) and phenobarbitone (PBT) on HLTE in mice using the maximum electroshock test.

4.4.2 Pentylene-tetrazole Induced Seizures in Mice

The HA stem bark extract did not protect mice against pentylenetetrazole induced seizures (Table 4.8). The latency to onset of seizure was also unaffected. However, the number of myoclonic body twitches was significantly ($p \leq 0.05$) reduced at all doses tested. 5 mg/kg produced a 27% decrease, while 10 and 20 mg/kg produced 59% and 61% reduction respectively compared with phenobarbitone (control) which produced a 95% decrease (Fig. 4.25). The mean number of myoclonic body twitches observed were 28.3±3.1 (5 mg/kg), 18.5±3.5 (10 mg/kg), 17.3±3.0 (20 mg/kg) and 2.0±0.4 (PHB, 30 mg/kg) compared to normal saline with 44.5±3.5 (Fig. 4.25).

Similarly the saponin fraction of the extract had no effect on sc-PTZ induced seizures (Table 4.9).

Table 4.8: Anticonvulsant effect of stem bark extract of *randia nilotica* (RNBE) and phenobarbitone (PBT) on sc-PTZ induced seizures in mice.

TREATMENT	PROTECTION	% PROTECTION
Normal Saline	0/6	0.00
RNBE 5 mg/kg	0/6	0.00
RNBE 10 mg/kg	0/6	0.00
RNBE 20 mg/kg	0/6	0.00
PBT 20 mg/kg	6/6	100.00

Results are presented as % protection. Phenobarbitone (PBT) showed 100% protection.

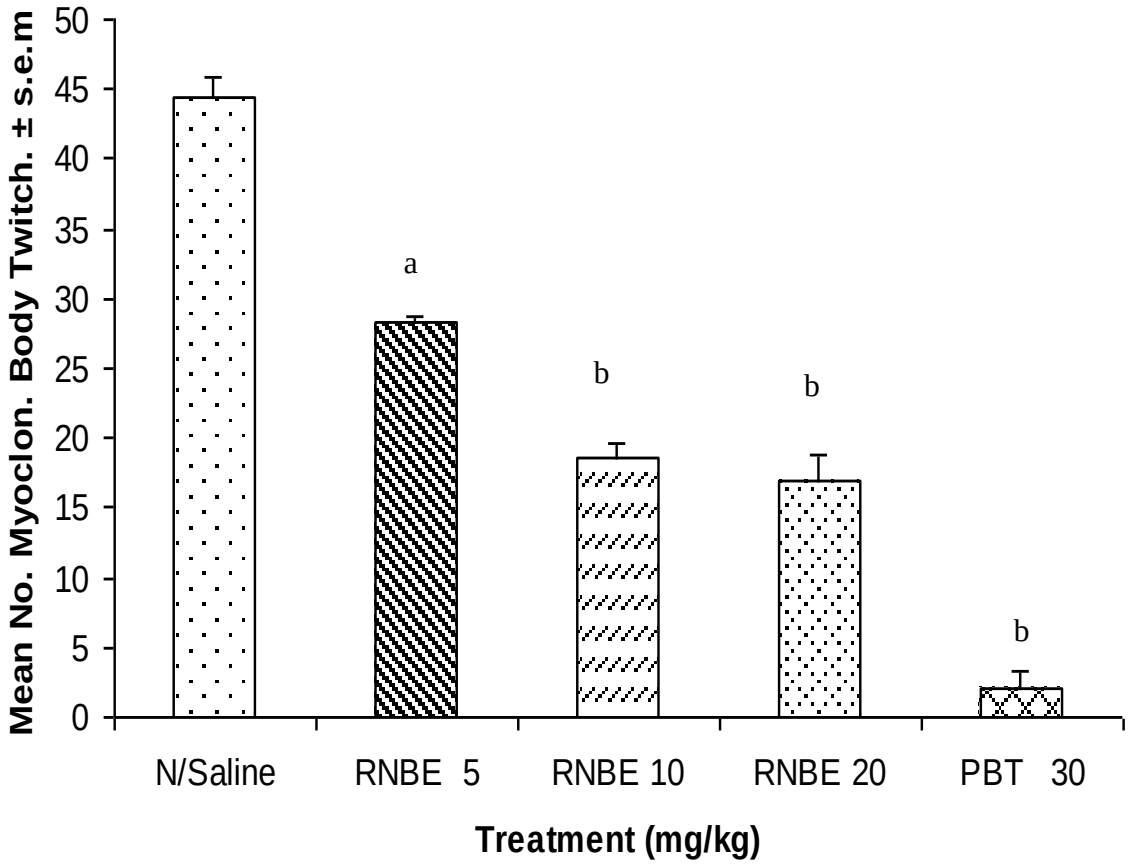


Fig. 4.25: Anticonvulsant effect (myoclonic body twitches) of the stem bark extract of *Randia nilotica* (RNBE) on sc-PTZ induced seizures in mice. Significant difference between control and treated groups: p≤ 0.05(a) and p≤ 0.001(b)-one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests. N=6 in each group.

Table 4.9: Anticonvulsant effect of saponin fraction of *Randia nilotica* (SFRN) stem bark extract on sc-PTZ induced seizure in mice.

TREATMENT	PROTECTION	% PROTECTION
CONT.(N/Saline)	0/6	0.00
SFRN 0.5 mg/kg	0/6	0.00
SFRN 1.0 mg/kg	0/6	0.00
SFRN 2.0 mg/kg	0/6	0.00
PBT 30 mg/kg	6/6	100.00

Results are presented as % protection. Phenobarbitone showed 100% protection.

4.4.3 Efect of extracts of *R. nilotica* on strychnine-induced seizures in mice:

The HA stem bark extract did not protect mice against strychnine-induced convulsions. The latency of tonic clonic seizures is also unaffected. Phenobarbitone (PBT) used as control had 100% protection (Table 4.10)

The saponin fraction of the extract similarly did not protect mice against strychnine-induced convulsions (Table 4.11)

Table 4.10: Anticonvulsant effects of stem bark extract of *Randia nilotica* (RNBE) and phenobarbitone (PBT) on STN-induced seizures in mice.

TREATMENT	PROTECTION	% PROTECTION
Normal Saline	0/6	0.00
RNBE 5 mg/kg	0/6	0.00
RNBE 10 mg/kg	0/6	0.00
RNBE 20 mg/kg	0/6	0.00
PBT 20 mg/kg	6/6	100.00

Results are presented as % protection. Phenobarbitone showed 100% protection.

Table 4.11: Anticonvulsant effects of saponin fraction of *randia nilotica* stem bark extract (SFRN) and phenobarbitone on STN-induced seizures in mice.

TREATMENT	PROTECTION	% PROTECTION
CONTROL(N/Saline)	0/6	0.00
SFRN 0.5mg/kg	0/6	0.00
SFRN 1.0mg/kg	0/6	0.00
SFRN 2.0mg/kg	0/6	0.00
PBT 30mg/kg	6/6	100.00

Results are presented as % protection. Phenobarbitone showed 100% protection.

4.5 Other Pharmacological Tests:

4.5.1 Effect of stem bark extract of *Randia nilotica* on tail suspension test in mice

The HA stem bark extract of *Randia nilotica* had no effect on duration of immobility time in mice, when subjected to tail suspension test in the doses tested (Fig.4.26). The doses tested produced an abnormal increase (though not significant) in duration of immobility time compared with control. Imipramine did not produce any decrease in duration of immobility time as well.

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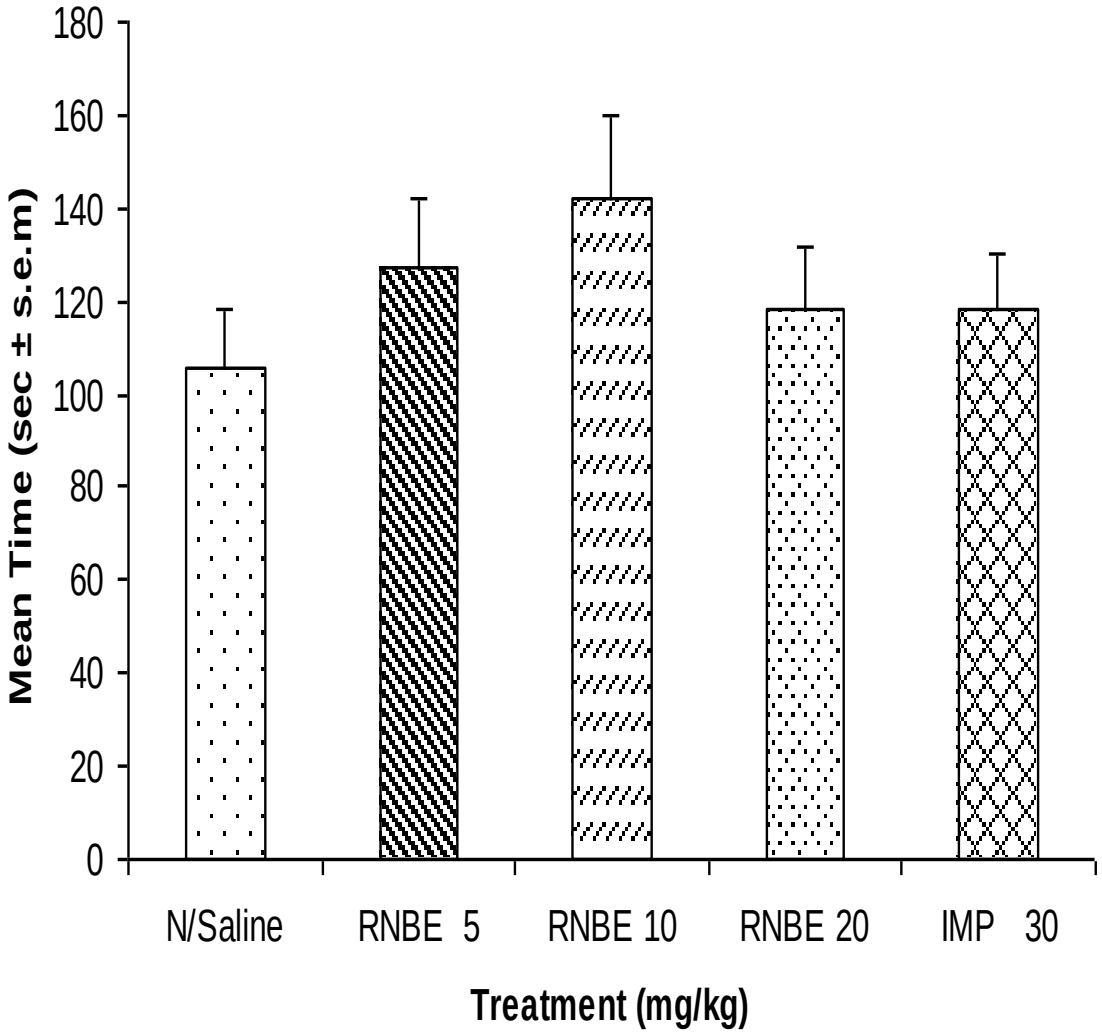


Fig. 4.26: ***Randia nilotica*** **stem bark extract (RNBE) and imipramine (IMP) and their effects on immobility time in tail suspension test in mice. No significant difference between control and treated groups: one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparison. N=6 in each group.**

4.5.2 Forced Swim Test in Rats

The HA stem bark extract of *Randia nilotica* (5, 10, 20 mg/kg body weight i.p.) dose-dependently diminished immobility time in rats when they were subjected to forced swim test. This reduction however, was not significant compared to control (Fig. 4.27).

The saponin fraction also diminished, but dose independently, duration of immobility time in rats. The decrease was significant ($p<0.05$) at 2.5 and 10 mg/kg compared with control (Fig. 4.28).

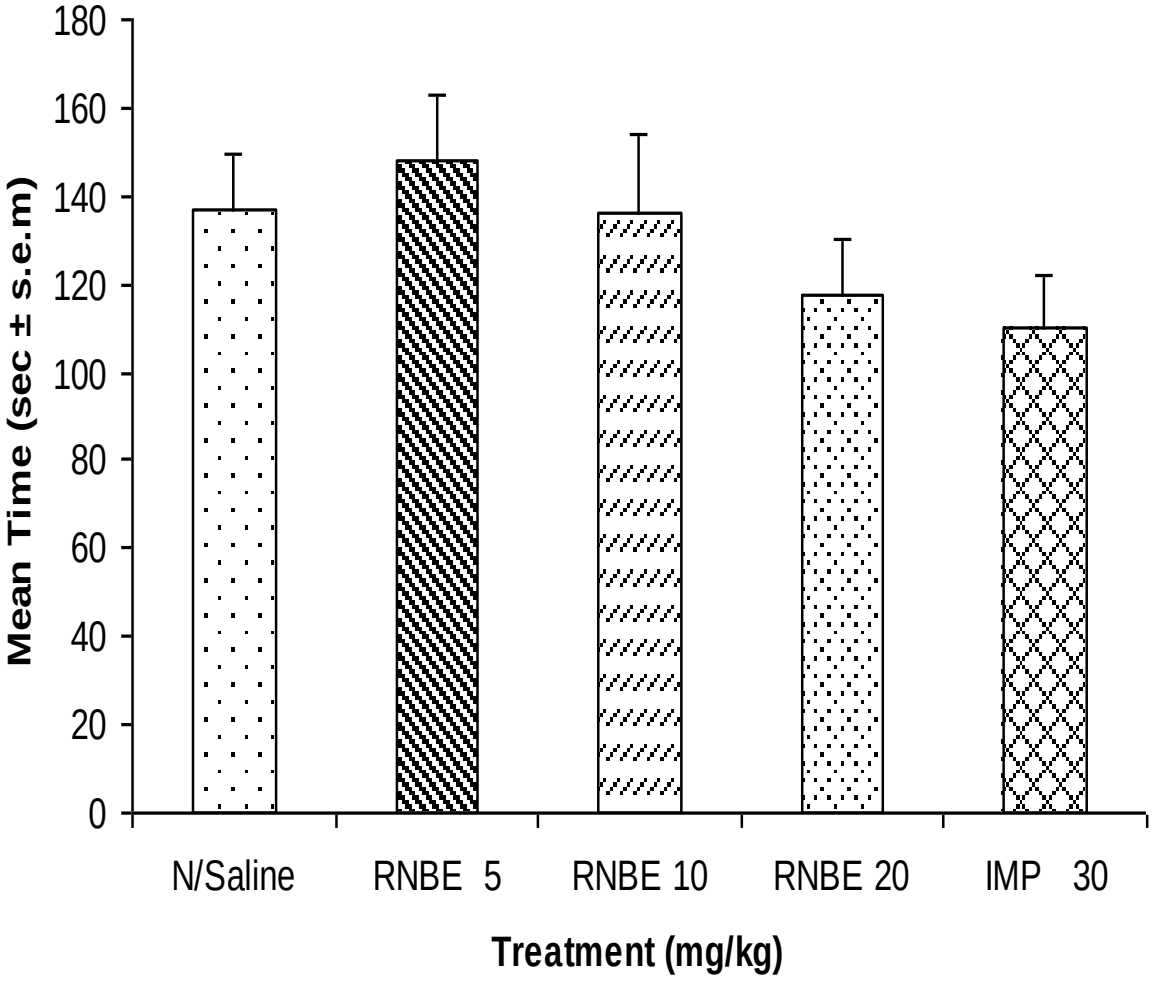


Fig. 4.27: Stem bark extract of *Randia nilotica* (RNBE) and imipramine (IMP) and their effects on duration of immobility time in forced swim test in rats. No significant difference between control and treated groups: one factor ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests for multiple comparison. N=6 in each group.

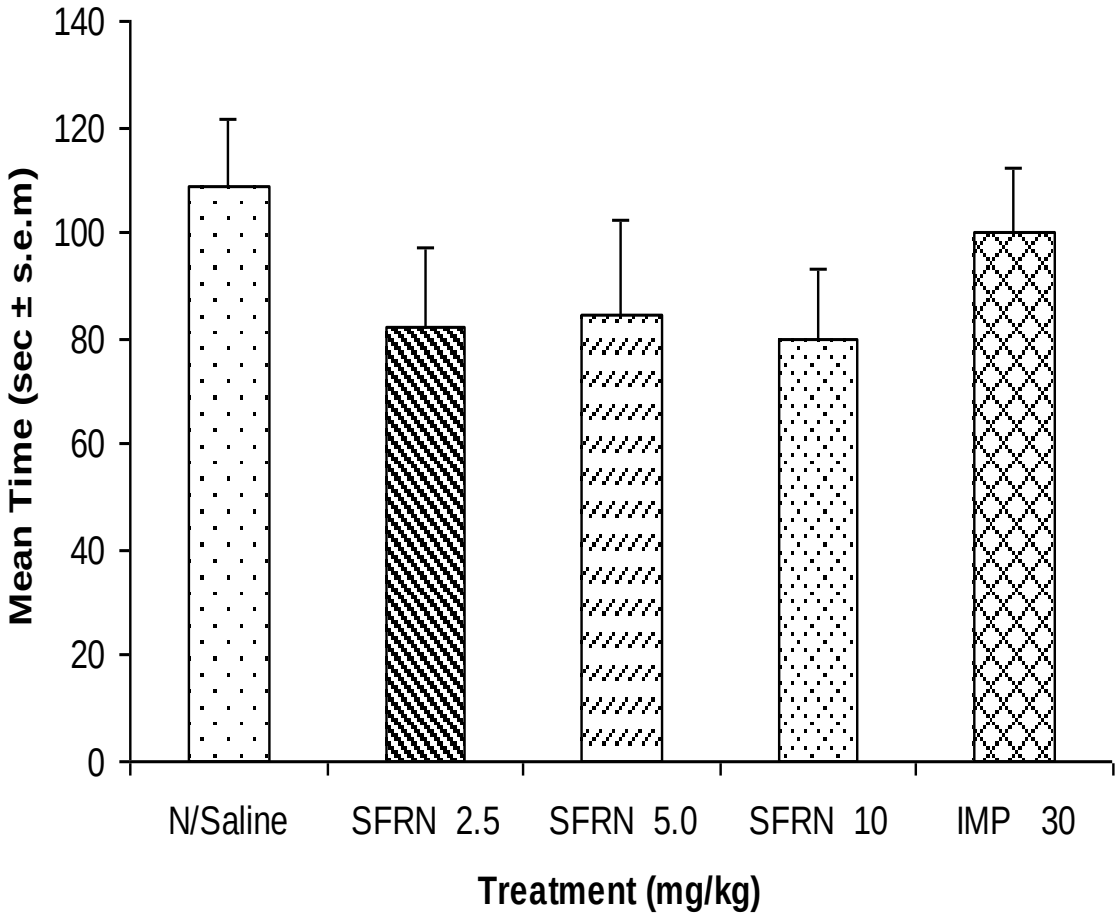


Fig. 4.28: Saponin fraction of *Randia nilotica* (SFRN) stem bark extract and imipramine (IMP) and their effects on duration of immobility time in forced swim test in rats. Significant difference between control and treated groups: p≤ 0.05(a)-one way ANOVA followed by DUNNET'S and SCHEFFE'S post hoc tests. N=6 in each group.

4.5.3 Results of EEG and EMG studies with RNBE

A biphasic activity wih the stem bark extract of *Randia nilotica* was observed in the EEG and EMG studies.The results outlined below were obtained 30 minutes after administration of the extracts.

The stem bark extract at 20 mg/kg body weight desynchronised the EEG of the hyperstriatum, optic tectum and of the pontine reticular formation. A slight increase in muscle activity was observed (Fig. 4.29).

At 40 mg/kg body weight, the extract also desynchronised the EEG of the hyperstriatum, optic tectum, and pontine reticular formation. It also increased the muscle activity of the rat (Fig. 4.30).

There was synchronisation of the EEG of the hyperstriatum and pontine reticular formation but not of the optic tectum at 80 mg/kg body weight (Fig. 4.31). A significant reduction in EMG activity was also observed.

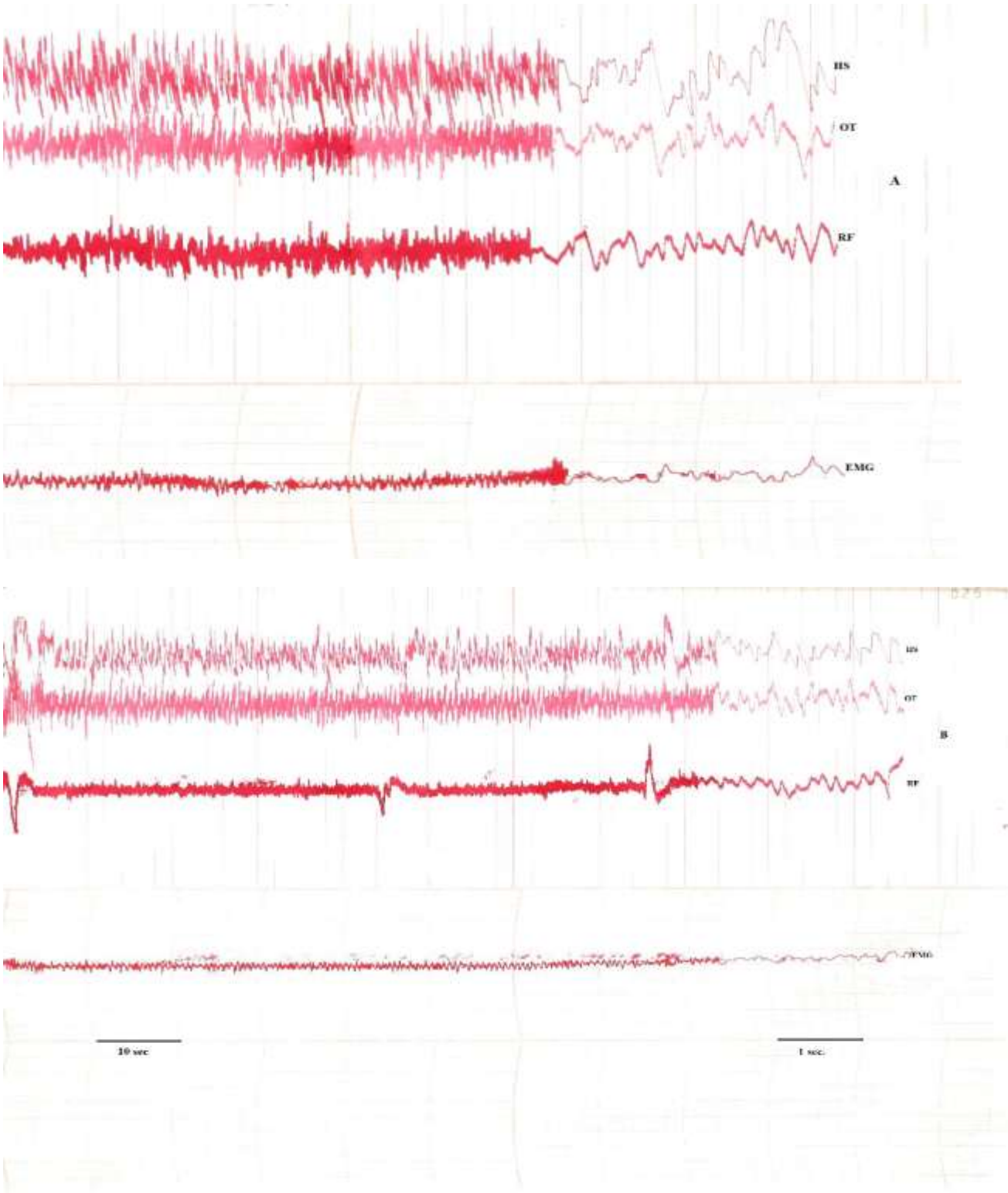


Fig.4.29: Effects of stem bark extract of *Randia nilotica* (20mg/kg) on rat EEG and EMG. Upper panels (A) are controls, lower panels (B) are extracts after 30 min. of treatment.

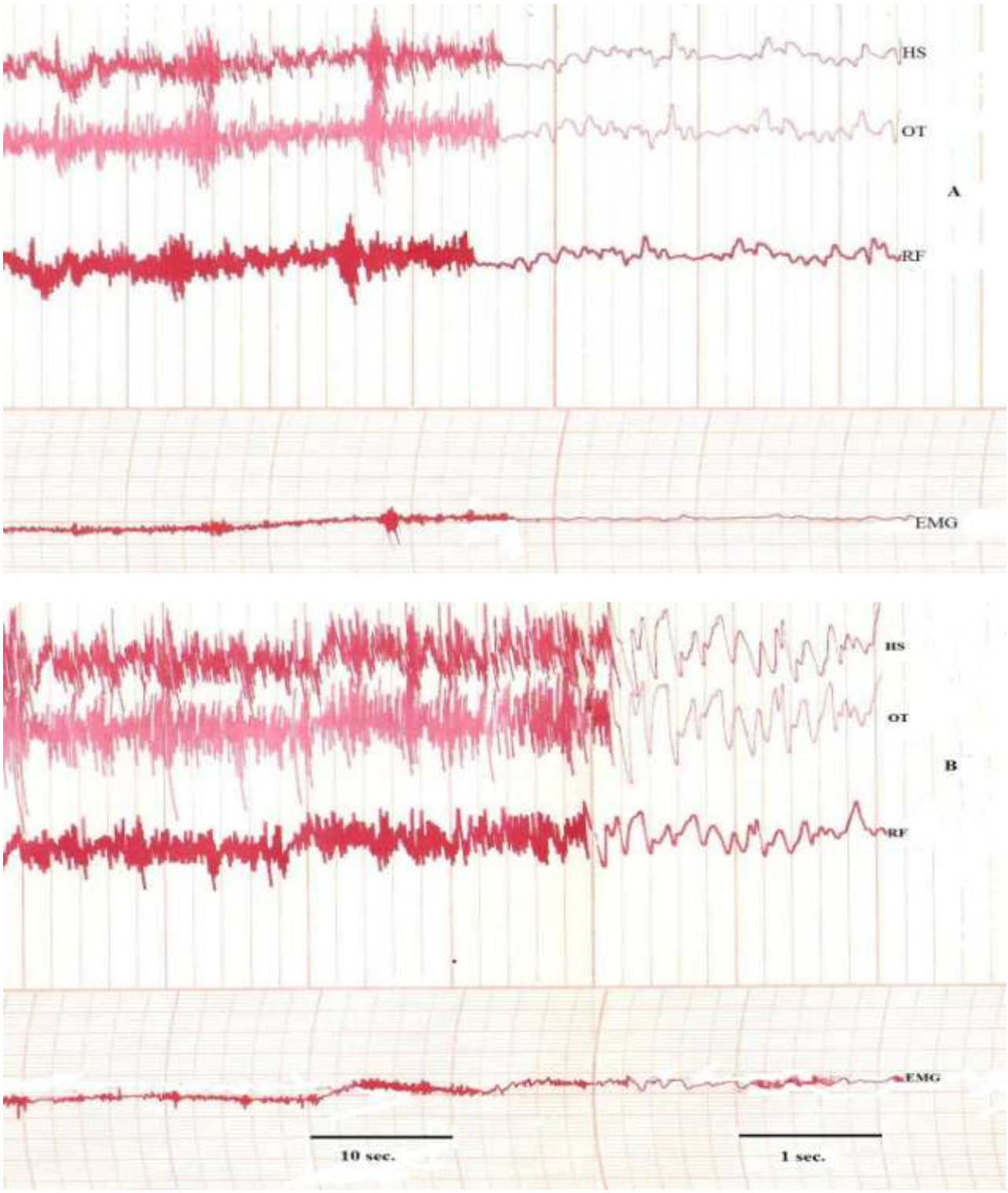


Fig.4.30: Effects of stem bark extract of *Randia nilotica* (40mg/kg) on rat EEG and EMG. Upper panels (A) are controls, lower panels (B) are extracts after 30 min. pre-treatment.

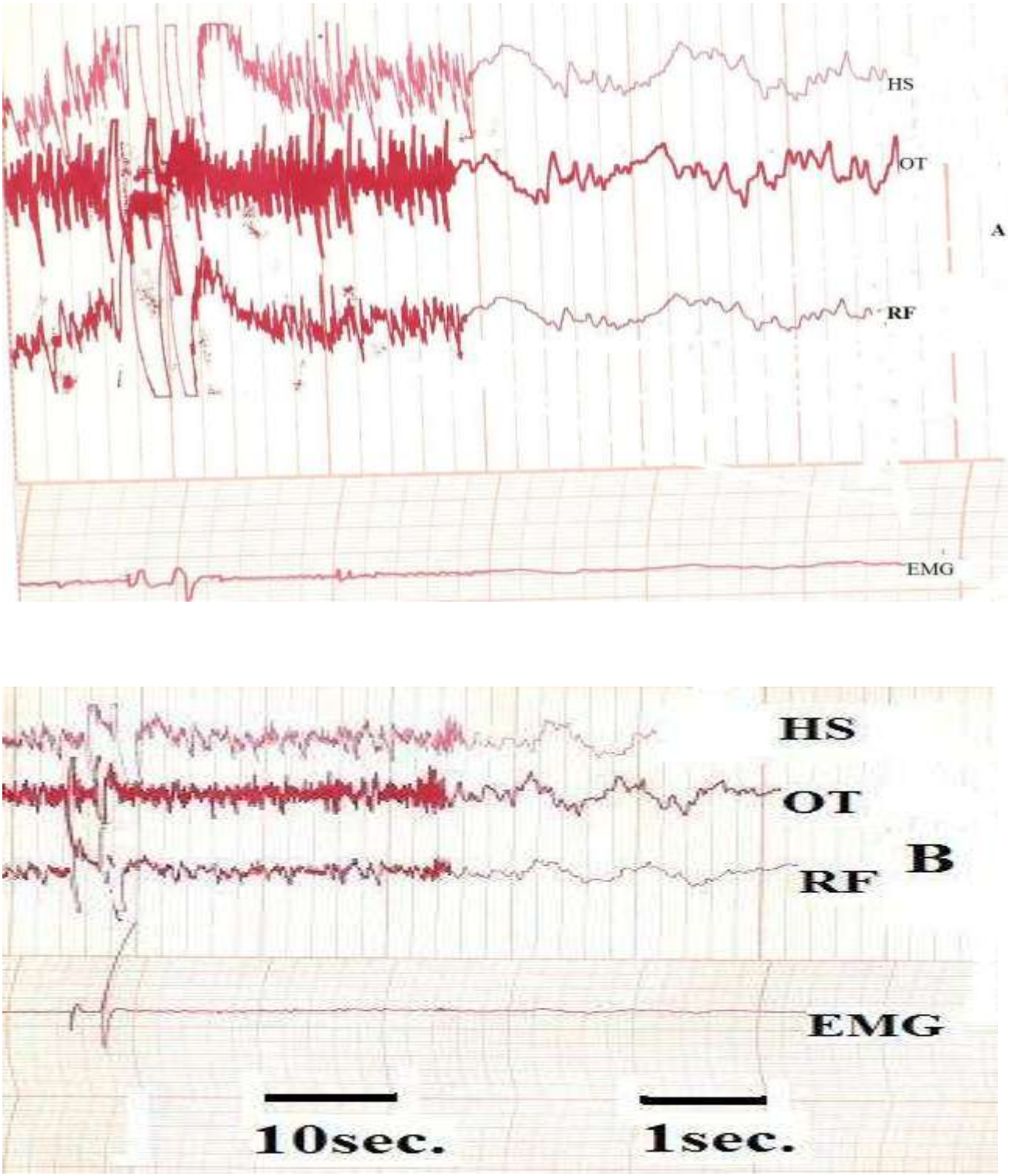


Fig.4.31: Effects of stem bark extract of *Randia nilotica* (80mg/kg) on rat EEG and EMG. Upper panels (A) are controls, lower panels (B) are extracts after 30min. pre-treatment.

4.5.4 Effect of SFRN on EEG and EMG of the rat

The saponin fraction of *Randia nilotica* (2.5 mg/kg) slightly synchronised EEG of the hyperstriatum, optic tectum and pontine reticular formation, with apparent reduction in EMG of the rat (Fig. 4.32).

At 5 mg/kg, synchronisation of the hyperstriatum, pontine reticular formation and optic tectum was observed. A reduction in EMG was also observed (Fig. 4.33).

10 mg/kg body weight of the extract did not produce any observable effect on the EEG of the hyperstriatum, pontine reticular formation and optic tectum as well as EMG activity throughout the period of observation (Fig. 4.34)

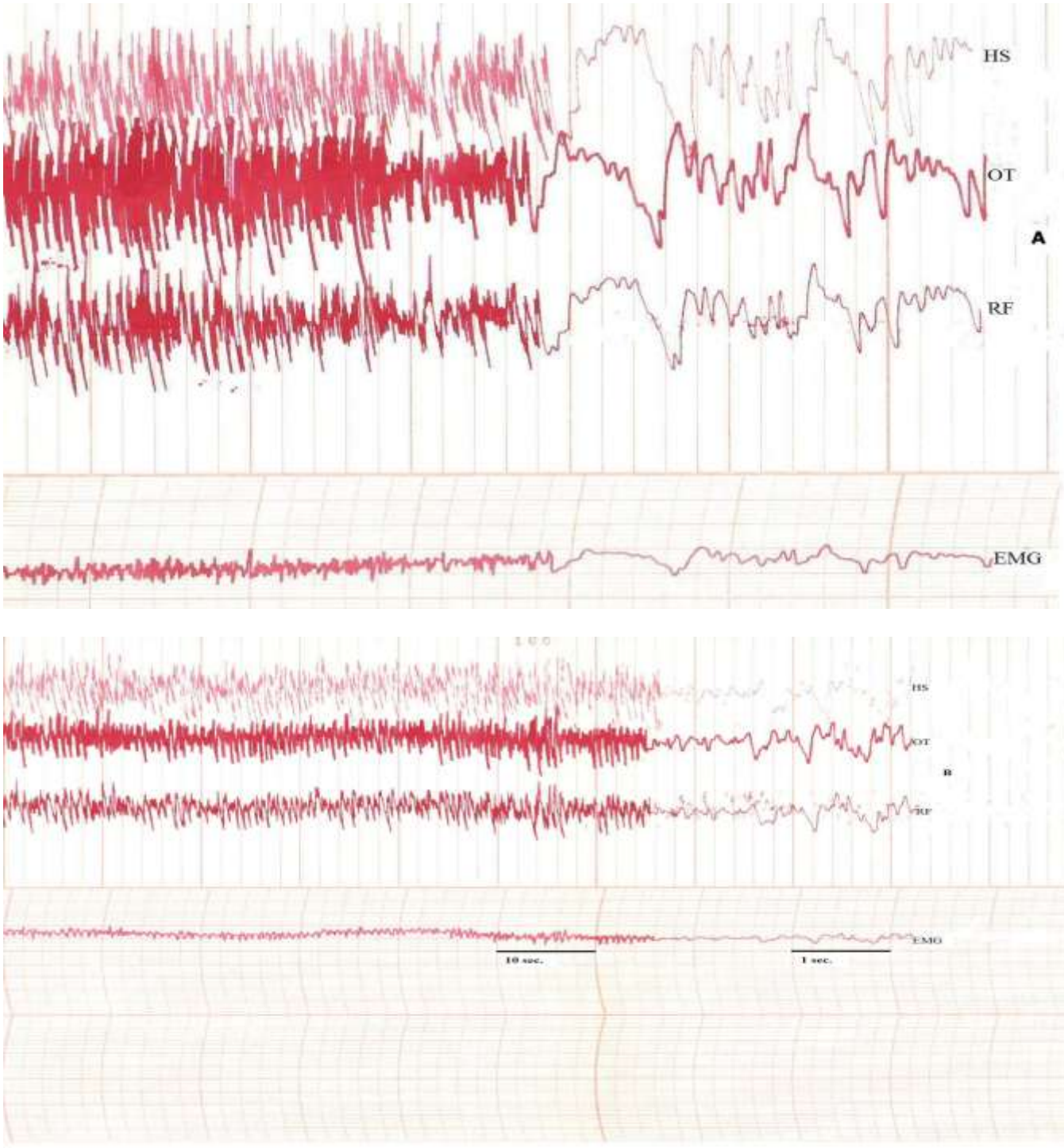


Fig.4.32: Effect of saponin fraction of *Randia nilotica* (2.5mg/kg) on rat EEG and EMG. Upper panels (A) are controls, lower panels (B) are extracts after 30 min. pre-treatment.

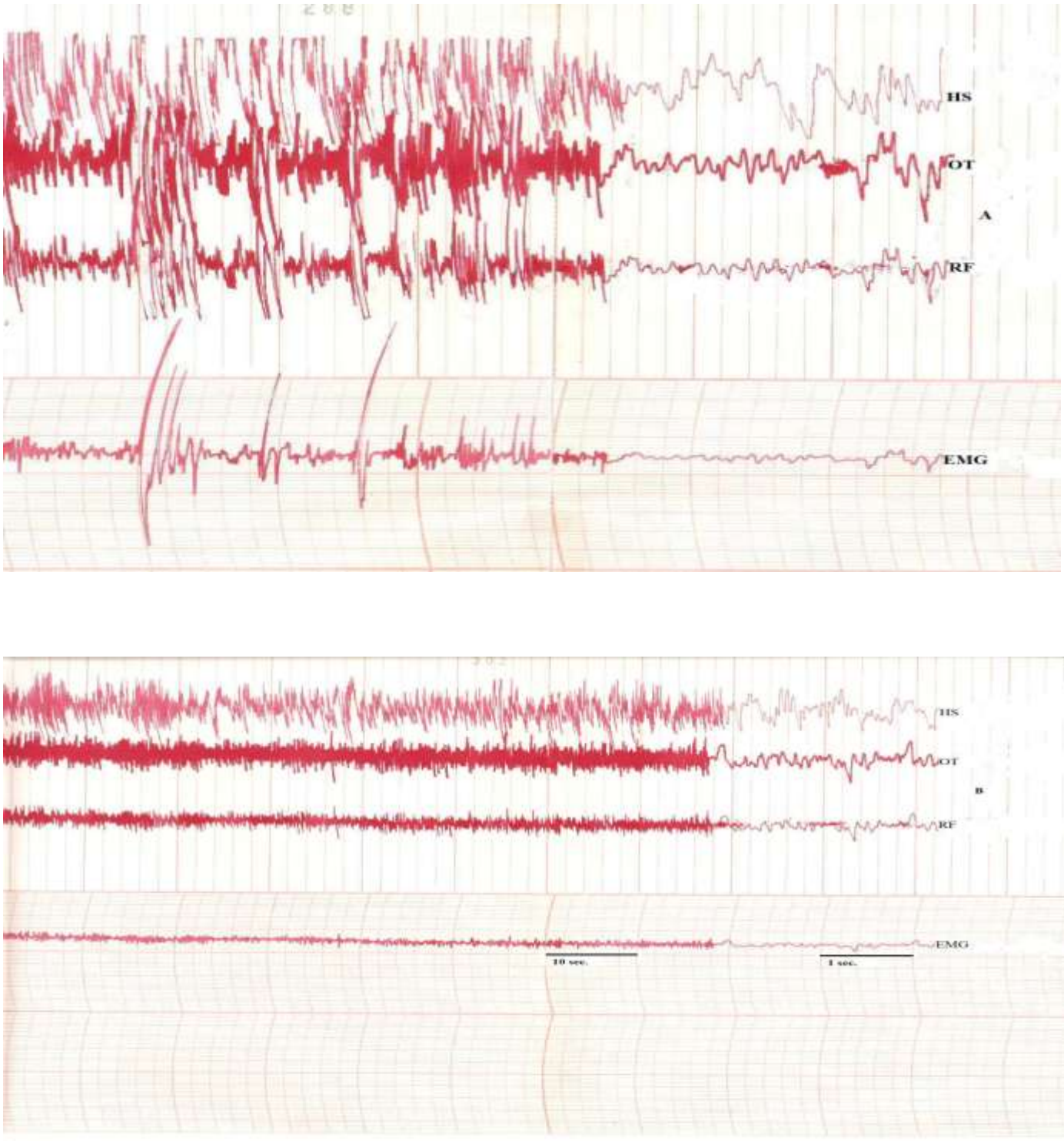


Fig. 4.33: Effect of saponin fraction of *Randia nilotica* (5 mg/kg) on rat EEG and EMG. Upper panels (A) are controls, lower panels (B) are extracts after 30 min. pre-treatment.

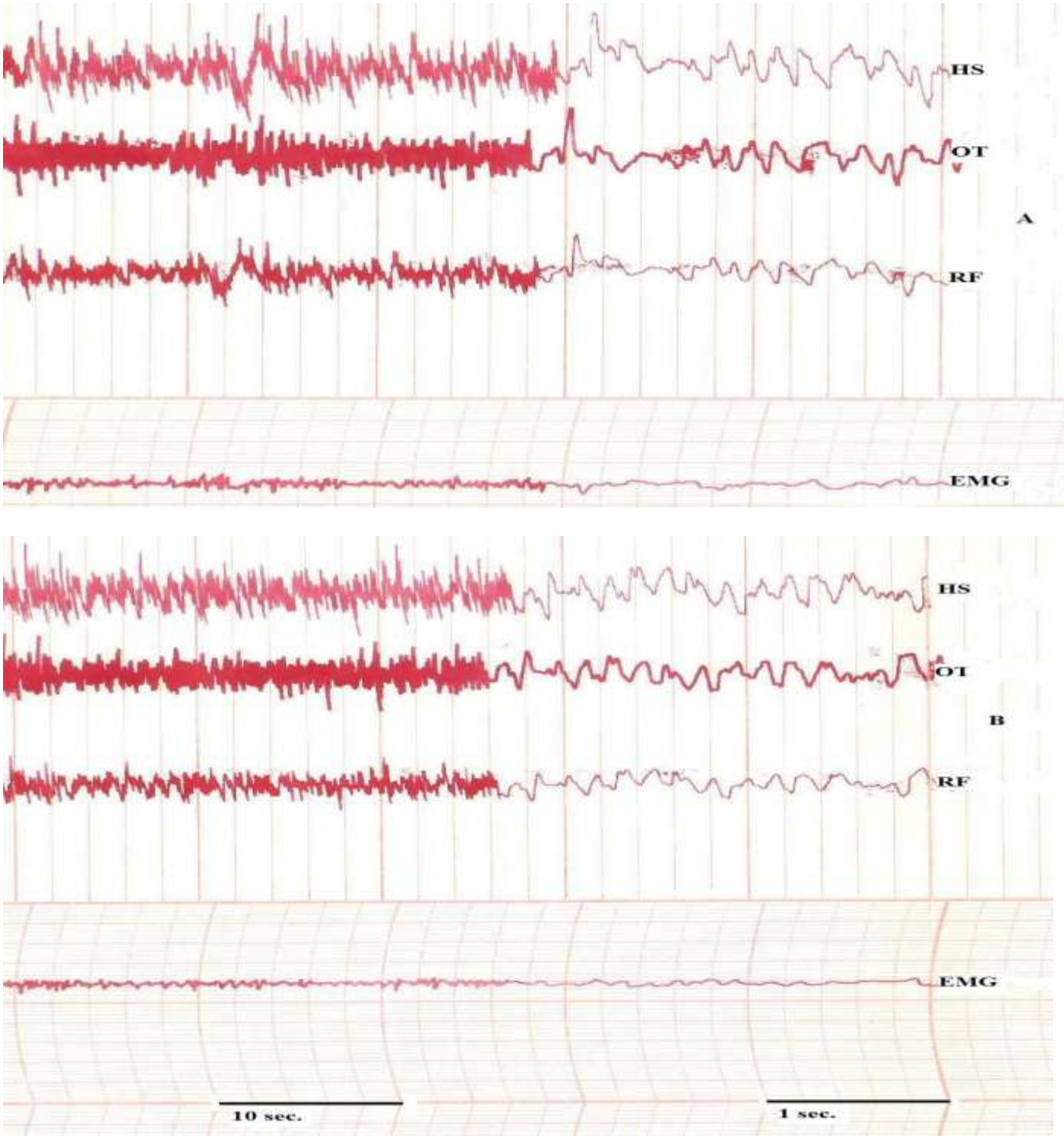


Fig. 4.34: Effects of saponin fraction of *Randia nilotica* (10 mg/kg) on rat EEG and EMG. Upper panels (A) are controls, lower panels (B) are extracts after 30 min. pre-treatment.

CHAPTER FIVE

5.0 DISCUSSIONS

The results obtained from this study, showed that, the stem bark of *Randia nilotica* stapf. contains substances with potential values in the treatment of neuropsychiatric and neurological diseases such as schizophrenia, anxiety and epilepsy.

Preliminary phytochemical screening of the stem bark revealed the presence of saponins, flavonoids, tannins and cardiac glycosides (Table 4.3). Similar findings were reported by El-Kheir and Salih (1980). Saponins, which were of the triterpenoid group, produced highest percent yield as compared to flavonoid or aqueous fractions (Table 4.4). It is known that saponins of triterpenoid group constitute majority of the saponins found in nature (Basu and Rastogi, 1967).

The acute toxicity studies conducted on the leaves, stem bark, root bark and secondary metabolites (saponins, flavonoids, aqueous fraction) indicate high toxicity of these compounds as evidenced in the low LD_{50} values of these extracts (Table 4.5). LD_{50} values however, are intended only to give an idea of how toxic a compound is, but not to reject toxic (unsafe) compounds and accept non-toxic (safe) ones (van Boxtel and Buitenhuis, 2001).

The diazepam-induced sleep was used as an index of CNS activity in this study. This test has been used to elucidate the CNS active properties of drugs (Turner, 1965b). Sedatives, hypnotics and tranquilisers as well as antidepressants are known to prolong diazepam induced sleeping time (Vogel and Vogel, 1997). Neuroleptics also prolong diazepam induced sleeping time while analeptics and stimulants shorten sleeping time (Vogel and Vogel, 1997).

The stem bark and leaf extracts produced the least reduction in onset of sleep when compared to the root bark extract (fig. 4.1, 4.3). The reduction in or rapid onset of sleep produced by the stem bark was at a dose 10 times less than that of the leaf extract. Similarly, the highest increase in duration of sleep was seen with the stem bark extract (Fig. 4.1, 4.7). The percentage yield of 18.4%, which was highest in stem bark (Table 4.1), could have contributed to the difference in activity..

Serotonin is a transmitter substance associated with the production of sleep and hence, stimulation of the raphe nuclei in the lower half of the pons and in the medulla causes almost natural sleep (Guyton and Hall, 2000). Sleep is reduced thus when there is a decrease in serotonin concentration or destruction of the dorsal raphe nucleous in the brain stem which contains most of the brains serotonergic cell bodies (Wauquier and Dugovich, 1990; Curtis and Jermaine, 2002).

For instance, reducing 5HT function with tryptophan depletion increases rapid eye movement (REM) sleep and decreases REM latency (Moore *et al*., 1998; Wilson *et al*., 2005), whereas promoting 5-HT function e.g. with the SSRI antidepressants greatly increase REM latency and reduced REM sleep (Wilson *et al*, 2005). There is increasing evidence from preclinical studies that the effect of the SSRIs are mediated via indirect stimulation of a 5-HT_{IA} receptor, probably in the REM generating area of the pons (Monti and Monti, 2000; Monaca *et al*., 2003).

Other neurotransmitter such as dopamine, norepinephrine, acetylcholine, GABA, histamine and neuropeptides (Muramyl peptide) have been suggested to play important roles in sleep

mechanisms (Guyton and Hall, 2000; Curtis and Jermaine, 2002; Gottessmann, 2002). In a separate study (unpublished), the extracts of *randia nilotica* were seen to attenuate acetylcholine-induced contractions on rabbit ileum.

Activation of central dopaminergic mechanism is implicated in both behavioural alertness and the waking process from pentobarbitone sleep in chicks (Osuide and Wambebe, 1980), while a decrease in dopamine receptor activity causes sleepiness (Gillin *et al*., 2000).
Increase firing of norepinephrine – containing neurones in the locus ceruleus on the other hand, will result in reduction of REM sleep and increased wakefulness (Curtiss and Jermaine 2002; Chindo, 2006). Acetylcholine and drugs that mimic its action increase occurrence of REM sleep (Guyton and Hall, 2000).

As far as the role of histaminergic system in sedation is concerned, there is considerable evidence that histamine is involved with the regulation of the sleep-wakefulness continuum (Pollard and Schwartz, 1987; Schwart and Arrang, 2002). A study in man with enantiomers of histamine H_1 receptor antagonists concluded that sedation can arise from H_1-antagonism alone (Nicholson *et al.*, 1991; Wilson *et al.*, 2005).

Furthermore, activation of $GABA_A$ receptor channel complex by the major inhibitory neurotransmitter GABA, in the CNS is known to favour sleep (Gottesmann, 2002). Neuropeptides (such as substance P and corticotrophin releasing factor) in the hypothalamus modulate neuronal activity during wakefulness (Culebras, 1992).

The effects of the hydroalcoholic extract of *Randia nilotica* on diazepam-induced sleep in mice may be attributed to their possible interaction with some of the aforementioned central

mechanism involved in the regulation of sleep. Most notably is the decrease in dopamine level which has been demonstrated in the amphetamine induced stereotypy test. On the other hand, the cholinergic involvement can be eliminated because of the ability of the extracts of *Randia nilotica* to abolish acetylcholine-induced contractions of rabbit ileum as stated above.

Fractions of the stem bark extract also possessed sedative effects, with the saponin fraction having the highest effect (Figs. 4.5, 4.6, 4.7 and 4.8). Alkaloids and saponins have been reported to show potent sedative activity (Wagner *et al*., 1983; Dubois *et al*., 1986, Taesotikul *et al*., 1998). Woo *et al.* (1980) also reported the sedative effects of saponins and flavonoids obtained from *ziziphus* seeds.

The saponin subfraction (SF_{1-4}) obtained after column chromatography also showed sedative effects (Figs.4.9, 4.10, 4.11, 4.12). These effects are prominent in SF_1 followed by SF_3 and then SF_2 (Fig. 4.13). The same argument outlined above may have been the reason for these effects.

The crude hydroalcoholic stem bark extract and saponin fraction dose dependently decrease number of head dips in the hole-board experiment (Figs. 4.14 and 4.15). This further supports claims that the plant contains substances that are sedative in nature (saponins). The hole-board test is a measure of exploratory behaviour in animals (File and Wardil, 1975). It has been accepted as an experimental model for the evaluation of psychotic, sedative and anxiety condition in animals (Crawley, 1985; Baldessarini, 1996). A decrease in number of head dips reveals a sedative behaviour (File and Pellow, 1985) and a high propensity for antipsychotic action (Feilding and Lal, 1978). Anxiolytics have been shown to increase number of head dips in the hole- board test (Takeda *et al*., 1998).

The hydroalcholic stem bark extract of *Randia nilotica* and its saponin fraction did not produce any observable effect on motor coordination in the treadmill experiment (Table 4.6, 4.7) suggesting that sedative effects are elicited via central mechanism and not due to peripheral neuromuscular blockade (Perez *et al*., 1998; Capaso *et al*, 1996).

According to Vogel and Vogel (1997), many central depressive drugs: benzodiazepines-diazepam, flurazepam, and neuroleptics-chlorpromozine, haloperidol are active in this test. The test also demonstrated lack of neurological impairment caused by these extracts, since it has been used to test for minimal neurotoxicity of a new compound (Stables and Kupferberg, 1997).

A more sensitive method of determining motor coordination deficit is the beam walking assay. The crude hydroalcoholic stem bark extract and its saponin fraction had no influence on time to reach goal box and on mean number of foot slips compared to control (Fig. 4.18, 4.19, 4.20 and 4.21). Diazepam, a benzodiazepine (BZD) which was used as control, showed significant increase in number of footslips compared with control and with the extracts tested.

The number of foot slips made during the beam walking assay is a more sensitive measure than the rotarod in detecting benzodiazepine-induced motor coordination deficits in mice, and may be more useful in predicting doses that cause sedation in the clinic (Stanley *et al*., 2005).

Diazepam does not significantly affect rotarod performance until approximately 72% receptor occupancy is achieved. By comparison, a significant deficit can be observed in the beam walking assay with approximately 32% receptor occupancy.

A statistically significant deficit can be observed at a lower GABA-A receptor occupancy level (i.e a lower level of pharmacological pertubation) for number of foot slips compared to time spent on beam or number of falls (Stanley *et al*., 2005).

The above findings signifies lack of motor deficit with these extracts even at relatively higher doses and that the extracts are not likely to cause clinical sedation at the doses tested (no ataxia was observed). Sedation has been a major limiting side effect in the use of benzodiazepines (Buffetjerrrot and Stewart, 2002).

The crude hydroalcoholic stem bark extract of *Randia nilotica* reduced significantly, jumping/climbing episodes in amphetamine-induced stereotype behaviour in mice, while sniffing and limb licking were reduced only at lower doses. An increase in sniffing and limb licking was observed at higher doses with the extract (Fig. 4.22).

Amphetamine is an indirectly acting sympathomimetic which causes release of catecholamines from neuronal storage pools. Its administration into rats or mice cause cessation of normal ratty behaviour and the appearance of a stereotyped behaviour – rearing, gnawing etc unrelated to external stimuli (Rang and Dale, 1993).

The release of dopamine from dopaminergic nerve terminals particularly at the striatal pathways and the mesolimbic system is widely believed to be responsible for the amphetamine-induced hyperactivity and stereotype behaviour (Van Rossom, 1970; Scheel-Kruger, 1971; Creese and Iversen 1974; Hoffman and Lefkowitz, 1996). These behavioural effects of amphetamine could be masked by conventional neuroleptics, most of which are

believed to be dopamine (D_2) receptor antagonists (Ljungberg and Ungerstedt, 1985; Anca *et al*., 1993).

Furthermore, 5-Hydroxytryptamine systems co-modulate other neurotransmitters, including the dopamine system in schizophrenia. There is little evidence however, relating specific problems in serotonin systems to symptoms of schizophrenia, although one possibility is the need for an appropriate balance between serotonin receptor subtype 1A (5-HT_{1A}) and serotonin receptor subtype 2(5-HT_2) functioning. Also, $5HT_3$ blockade reduces dopamine receptor subtype 2 (D2)-induced movement disorders common with traditional antipsychotics. The 5-HT_2 blockade may also improve negative symptoms by relieving frontal cortex hypofunctioning.

In addition, glutamate is involved in nearly all functions that are disrupted in schizophrenia. N-methyl-D-aspartate (NMDA) receptors, a type of glutamate receptors is blocked by phencyclidine (PCP) which causes a schizophrenic-like picture of hallucinations, delusions, negative symptoms and cognitive changes, often unresponsive to D_2-antagonists. NMDA receptor hypofunctioning can also lead to dopamine receptor upregulation and can itself cause brain damage.

Aripiprazole, a novel antipsychotic exhibits a pharmacological profile that is different from other atypical antipsychotics, with antagonism at D_2 dopamine receptors in the mesolimbic pathway and a partial agonist activity at D_2 receptors in the mesocortical pathway (Goodnick and Jerry, 2002; Salmoiraghi and Odiyoor, 2006). It also displays partial agonism at the 5-HT_{1A} receptors and antagonism at 5-HT_{2A} receptors (Davies *et al*., 2004; De Leon *et al*., 2004).

The stem bark may have had antagonism at some receptors sites (e.g. mesolimbic) and agonism at other receptor sites (e.g. nigrostriatal), especially at different dose levels that is why it has a biphasic effect in this test.

Protection against hind limb tonic extension (HLTE) in the maximum electroshock test (MEST) projects anticonvulsant activity of antiepileptic drugs (AEDs) that prevents the spread of the epileptic seizure discharge from an epileptic focus during seizure activity (Raza et al., 2001). Inhibition of HLTE is the common feature of maximal electroshock in rodents, cats, monkeys and humans and the response of rodent's brains to the anticonvulsants is similar to that of humans (Swinyard, 1972). There are no false negatives in the MEST and all the currently available AEDs that are clinically effective in the treatment of generalised tonic-clonic and partial seizures such as phenobarbitone (PBT) carbamazepine, (CBZ) oxcarbazepine and lamotrigine also suppress HLTE in MEST (Browning, 1992; Kupferberg and Schmutz, 1998; Rho and Sankar, 1999; Rogawski and Porter, 1990). Protection against HLTE also indicates the ability of a testing material to inhibit or prevent seizure discharge within the brainstem seizure substrate (Browning, 1992).

The strong inhibitory effect of the hydroalcoholic stem bark extract of *Randia nilotica* (Fig. 4.23) and of its saponin fraction (Fig. 4.24) suggests that these extracts possesses potent anticonvulsant activity for treatment of generalised tonic clonic and partial seizures.

Pentylenetetrazole (PTZ) is a known convulsnat and therefore anticonvulsant activity in the subcutaneous (SC) PTZ test identifies compounds that can raise the seizure threshold in the brain (White *et al*., 1998). AEDs effective in the therapy of genralized seizures of (absence

myoclonic) petit mal type such as PBT, valproic acid (VPA), ethosuximide (ETX) and benzodiazepine (BZD) exhibit dose-dependent suppression of various seizure patterns induced by PTZ (Loscher *et al.,* 1991b). However, some antiepileptic drugs do not show PTZ antagonism at tolerable doses (Lippa *et al*., 1979).

At the cellular level, one of the basic mechanisms of actions of AEDs such as ETX and VPA is the suppression of T-type calcium currents in thalamic neurons (Rho and Sankar, 1999; Meldrum, 1996; Macdonal and Kelly, 1994).

PTZ has also been shown to interact with the GABA neurotransmitter and the GABA-receptor complex (Doctor *et al.,* 1982; Bum *et al.,* 2001). Standard antiepileptic drugs such as Diazepam and PBT are thought to produce their effects by enhancing GABA-mediated inhibition in the brain (Macdonald and Kelly, 1994; Rogawski and Porter, 1990).

A large body of electrophysiological and biochemical data also suggested the involvement of dopaminorgic mechanism in PTZ-induced convulsions (Jones and Robert, 1968; Dadkar *et al.,* 1979; Amabeoku, 1989). These workers observed that dopamine reduces the threshold of PTZ convulsions in mice and pimozide (a specific dopamine receptor antagonist) protected experimental animals against PTZ-induced seizures (Chindo, 2006).

The absence of anticonvulsant activity in the sc-PTZ induced seizure test (Table 4.8, 4.9) suggest that, the compounds present in the stembark of *Randia nilotica* does not suppress calcium currents in the thalamic neurones, or interact with the GABA receptor complex or with GABA-neurotransmiter. It is not clear however whether the doses of the extracts used

are a factor, since there was significant reduction in total number of myoclonic jerks compared to control (Fg. 4.26).

Strychnine (STN) is a competitive antagonist of the inhibitory amino acid glycine (Larson, 1969). The absence of anticonvulsant activity in the sc-STN test (Table 4.10, 4.11), suggests that the compounds in the crude and saponin fraction do not interact with the glycine receptors.

Forced swim (Porsolt *et al.,* 1985) has gained considerable acceptance as reliable screens for depression. The immobility displayed by rodents when subjected to unavoidable and inescapable stress has been hypothesized to reflect behavioural despair which in turn may reflect depressive disorders in humans. Clinically effective antidepressants reduce the immobility time displayed by rodents after active and unsuccessful attempts to escape during forced swim or when suspended by the tail. The hydroalcoholic stem bark extract and its saponin fraction, dose-dependently diminished immobility time when subjected to the forced swim test (Fig. 4.25). This suggests that the extract may have some antidepressant activity. There was no observable effect however in the tail suspension test which may be due to the fact that certain strains of mice are essentially resistant to this screening method (Vogel and Vogel, 1997).

Depression results from functional deficiency of monoamines, such as norepinephrine and serotonin at certain sites (hippocampus, striatum, amygdale, cortex) in the brain (Howland and Mycek, 2006). Most clinically useful antidepressant drugs therefore, potentiate directly or indirectly the actions of norepinephrine and/or serotonin in the brain. Selective serotonin re-uptake inhibitors (SSRIs) specifically block re-uptake of serotonin, leading to increased

concentrations of the neurotransmitter in the synaptic clefts and ultimately, to greater postsynaptic neuronal activity. In contrast, the tricyclic antidepressants (TCAs) non-selectively inhibit re-uptake of norepinephrine and serotonin into presynaptic nerve terminals. By blocking the major route of neurotransmitter removal, the TCAs lead to increased concentrations of monoamines in the synaptic cleft ultimately resulting in antidepressant effects (Howland and Mycek, 2006). Similarly, the serotonin/norepinephrine re-uptake inhibitors inhibit re-uptake of both serotonin and norepinephrine and are effective where SSRIs fail.

On the other hand, the monoamine oxidase inhibitors (MAOIs) reversibly or irreversibly inactivate the enzyme, permitting neurotransmitter molecules to escape degradation and therefore, to both accumulate within the presynaptic neurone and leak into the synaptic space. This causes activation of norepinephrine (NE) and serotonin receptors, and is responsible for the antidepressant action of some drugs (Howland and Mycek, 2006).

Furthermore, accumulative evidences over a decade demonstrate that, the brain glutamic acid accumulation as well as its excitotoxicity also play an important role in depressive disorder (Skolnick, 1999). Therefore, inhibition of N-methyl-D-aspartate (NMDA) receptor function appears to be one of the common actions for antidepressants (Li *et al.*, 2006). NMDA receptor antagonists such as MK 801, amantadine or Zn^{2+} posses antidepressant-like effects in animal models (Kroczka *et al.*, 2000; Petrie *et al.*, 2000). NMDA receptors are ligand gated Ca^{2+} channels with varying subunits, whose activation leads to Ca^{2+} influxes. The overloading intracellular Ca^{2+} ($[Ca^{2+}]$) will activate downstream nitric oxide synthase (NOS), which catalyses L-arginine into nitric oxide (NO), Ca^{2+} and NO

and are both important signal messages responsible for NMDA receptor function. It has been demonstrated that NOS inhibitors posses antidepressant-like effect in animal models (Harkin *et al*., 1999).

The observable effects of the crude extract and its saponin fraction on forced swim test may be due to one of the aforementioned mechanisms, most probably through inhibition of re-uptake. Drugs which increase synaptic noradrenaline content seem to reduce immobility time (Porsolt *et al*., 1979). However it is not clear which adrenoceptor mediates this effect, since ß-stimulants are inactive (Porsolt *et al*., 1979; Kitada *et al*., 1983) as both clonidine and yohimbine, agonist and antagonist of alpha-$_2$ adrenergic receptors respectively, reduced immobility time (Porsolt *et al*., 1979).

Results of EEG and EMG studies with the stem bark of *randia nilotica* (Figs. 4.29, 4.30, 4.31) and of its saponin fractions (Figs. 4.32, 4.33, 4.34) indicated strong sedative effects of these extracts. Synchronisation was observed with the extracts particularly saponins at all doses tested and crude extract at 80 mg/kg. Such data indicate electrocortical sleep/depression (Anuka *et al*., 2001) and agrees with the behavioural sleep/depression observed in some of the studies carried out (hole-board, pentobarbitone-induced sleep e.t.c.). The waves were high voltage, beta (> 14 cycles per second) typical of rapid eye movement (REM) sleep (Osim, 2003). Muscle tonus was almost absent in some instances. Desynchronisation of the EEG of HS, RF and OT and weak activation of the EMG as observed with the extracts at certain periods in the course of the study indicate electrocortical stimulation (Anuka et al., 2001). And this explains the biphasic nature of the extracts of *randia nilotica* as observed in certain tests conducted particularly, amphetamine-induced stereotype behaviour in mice.

6.0 CHAPTER SIX

SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

The overall results of this study revealed that, the extracts of *Randia nilotica* posses good sedative activity compared to standard drugs used in most tests conducted. Potentiation of pentobarbitone and diazepam induced sleep as well as reduction in mean number of head dips by the crude extract and its saponin fraction strongly suggest depressant effect of *Randia nilotica*. This effect appears to be centrally mediated and not due to peripheral neuromuscular blockade. This is evidenced by the inability of the extract to produce any observable effect on motor coordination in the treadmill test or the beam walk assay. The hypothesis of central activity was further supported by the ability of the extract to attenuate jumping/climbing and to some extent sniffing and limb licking in the amphetamine induced sterotype behaviour in mice. Synchronization of the EEG of the rat by HA stem bark extract and its saponin fraction is by far the most important evidence of the central depressant effect of the extracts as well as their inhibition of HLTE in the MES test. Phytochemical analysis has shown that saponins contribute more to the sedative effect of the extracts. These effects may be beneficial in anxiolysis, antipsychoses muscle relaxant and anticonvulsant effects.

The major inference that could be drawn from the above findings is in their support for the uses of *Randia nilotica* in traditional medicine in treatment of neuropsychiatric (psychosis) and neurological disorders (epilepsy). It can also be inferred, that the extracts of *Randia nilotica* are relatively safe at the doses tested and with little tendency to cause clinical sedation. I therefore conclude that, *Randia nilotica* merits further attention in the search for newer agents for the treatment of neuropsychiatric and neurological diseases.

However it is necessary to carry out repeated column chromatography with subfraction of the saponin fraction of *Randia nilotica* with a view to purify and obtain a novel lead molecule. More pharmacological assay technique such as the 5-HT binding assay, should also be employed to ascertain the exact mechanism of action of the novel lead molecule. Finally, sub-acute and chronic toxicity studies should be conducted to ensure the overall safety of the new compound.

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