NEUROBEHAVIOURAL EFFECTS OF HYDRO-METHANOL LEAF EXTRACT OF *Bryophyllum pinnatum* IN MICE

BY

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DECLARATION

I declare that this thesis titled 'NEUROBEHAVIORAL EFFECTS OF HYDRO-METHANOL LEAF EXTRACT OF *BRYOPHYLLUM PINNATUM* IN MICE' has been performed by me in the Department of Human Physiology under the supervision of Prof. Dr. M.A. Mabrouk and Dr. Yusuf Tanko.

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

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CERTIFICATION

This thesis titled "Neurobehavioral Effects Of Hydro-Methanol Leaf Extract Of *Bryophyllum pinnatum"* by Momoh, Ibrahim Joseph meets the regulations governing the award of the degree of Master of Science of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to the memory of my father, late Mal. Momoh Akpa and my mother, Mrs Ramatu Momoh for their unwavering love, support and prayers.

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ABSTRACT

There are thousands of plants that are used traditionally in Africa for the management of anxiety disorders, convulsion, motor disturbances and sleep disorders. However, only a few have received scientific scrutiny. This study was carried out to evaluate the effect of the hydro-methanol leaf extract of *Bryophyllum pinnatum* (family: *Crassulaceae*) in some neurobehaviours in mice weighing between 25-30g at doses of 25, 50 and 100mg/kg. The parameters evaluated were the hole board test for explorative activity, beam-walking assay for motor coordination deficit, diazepam-induced sleeping time and Pentylenetetrazole (PTZ)-induced convulsion. The results revealed that the extract showed a statistical significant ($p<0.05$) increase in the number of foot slips and beam latency at 25 and 50mg/kg. It also showed a decrease in head dips but was not significant. It also revealed a statistical significant ($p<0.05$) decrease in the onset of sleep and a statistical significant ($p<0.05$) prolongation of sleep time in a dose dependent manner. The extract also showed a biphasic activity by delaying the onset of convulsion and increasing protection at the doses of 25 and 100mg/kg in mice. The LD_{50} of the extract was calculated to be 400mg/kg. These results showed that the extract possesses some central nervous system (CNS) depressant activity which may be due to the effect or activity of one or more of the phytochemicals present in the plant.

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LISTS OF ABBREVIATIONS/SYMBOLS

5-HT	-	Serotonin
AASM	-	American Academy of sleep medicine
ADH	-	Antidieuretic Hormone
ADNFLE	-	Autosomal Dominant Nocturnal Frontal Lobe Epilepsy
BDZ	-	Benzodiazepines
BF	-	Basal Forebrain
BOEC	-	Benign Occipital Epilepsy of Childhood
CAE	-	Childhood Absence Epilepsy
CCK	-	Cholecystokinin
CE	-	Catamenial Epilepsy
CMC	-	Cingulate Motor Cortex
CNS	-	Central Nervous System
DoF	-	Degree of Freedom
DR	-	Dorsal Raphe
EEG	-	Elecro-encephalogram
EMG	-	Electro-myogram
EOG	-	Electro-oculogram
GABA	-	Gamma-Amino Butyric Acid
GI	-	Gastro intestinal
Hcrt	-	Hypocretin
HKB	-	Haken Kelso and Bunz
H_o	-	Null hypothesis
ILAE	-	International League Against Epilepsy
LC	-	Locus Coereleus

LDT	-	Lateraldorsal Tegmental Nucleus
MAO	-	Mono-amine oxidase
MEST	-	Maximal Electro-Shock Test
NREMS	-	Non-Rapid Eye Movement Sleep
OX	-	Orexin
PCO_2	-	Partial pressure of carbon dioxide
PMC	-	Premotor Cortex
PO_2	-	Partial pressure of oxygen
PPT	-	Pedunculopontine
PTZ	-	Pentylenetretrazole
REMS	-	Rapid Eye Movement Sleep
RE	-	Thalamic Reticular Nuclear Complex
SD	-	Sleep Deprivation
SIDS	-	Sudden Infant Death Syndrome
SMA	-	Supplementary Motor Cortex
SWS	-	Slow Wave Sleep
TC	-	Thalamocortical
TM	-	Tuberomammary Nucleus
VLPO	-	Ventrolateral Preoptic Nucleus
VTA	-	Ventral Tegmental Area
WHO	-	World Health Organization

CHAPTER ONE

2.0 INTRODUCTION

In developing countries, remedies from plants play an important role in the health care of millions of people. Despite immense technological advancement in modern medicine, many people in developing countries still depend on traditional healing practices and medicinal plants for their daily health care needs (Ojewole, 2005). Traditional medicine involves the use of herbal medicine, animal parts and minerals. However, herbal medicines are the most widely used of the three. It is estimated that about 80% of the world population is dependent (wholly or partially) on plant-based drugs (WHO, 1996). In most developing countries of the world, including Nigeria, people of all classes depend heavily on herbal medications for the management of various diseases despite the availability of modern medicine (Nwabuisi, 2002).

All cultures have traditions of folkloric medicine that include the use of plants and other ethnopharmacological products. Despite availability of modern medicine in the treatment of different ailments, people still continue to patronize herbal remedies for obvious reasons of availability and affordability. Different medicinal plants have different medicinal properties. No one herb is found to be used just for one purpose (Ghasi *et al*., 2011).

One important area in which herbal medicines enjoy high patronage worldwide is in the management of nervous system disorders. A good number of natural product scientists believe that the initial selection of plants with diverse application in traditional medicine might be encouraged by their easily noticeable central nervous system (CNS) effects (Amos *et al*., 2001).

The importance of medicinal plants in traditional healthcare practices, providing clues to new areas of research and in biodiversity conservation is now well recognized. The use of

traditional medicine and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed (Ranjan *et al.,* 2010). The search for new pharmacologically active agents obtained by screening natural sources such as microbial fermentations and plant extracts has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases (Kumar *et al.,* 2009).

Traditional healing practices and the use of medicinal plants still constitute a major source of therapy and health care needs in Africa. Medicinal plants are distributed worldwide, but they are most abundant in tropical countries (Calixto, 2000; Lewis, 2001). The vegetation and floral biodiversity of Africa provide African traditional healers with an impressive pool of 'natural pharmacy' from which plants are selected as remedies or as ingredients to prepare herbal medicines for a plethora of human and veterinary disorders (Ojewole, 2005). One of such frequently used African medicinal plant is *Bryophyllum pinnatum* (family: *Crassulaceae*).

1.1 Ecology and Distribution of *Bryophyllum pinnatum*

Bryophyllum pinnatum (*B. pinnatum)* is a weed of gardens, parks, roadsides, railway lines, waste areas, coastal environs, creek-banks, open woodlands, forests and forest margins. It is a garden plant that is naturalised in tropical, sub-tropical and warmer temperate regions

It is relatively common in the coastal regions of central and northern Queensland, present in other parts of New South Wales, and naturalised on several offshore islands (i.e. Lord Howe Island, Norfolk Island and the Cocos Islands).

It is widely naturalised in other parts of the world including tropical eastern Africa, Asia (e.g. Taiwan, Indonesia and New Guinea), New Zealand, south-eastern USA (i.e. Florida), the Caribbean, and the Pacific (i.e. the Galapagos Islands, Melanesia, Polynesia and Hawaii) (Biosecurity Queensland, 2011).

Classified as a weed (Oliver-Bever, 1983), the plant flourishes throughout the Southern part of Nigeria (Gill, 1992).

1.2 Taxonomy

The plant belongs to the kingdom plantae with the family crassulaceae. Its generic name is kalanchoe belonging to the species *K. Pinnata* (Paranjpe, 2005).

1.3 Plant Description

Bryophyllum pinnatum is a fleshy shrub, which grows 2-4 feet tall. The plant, which belongs to the family *Crassulaceae* is widely grown in the region for ornamental and medicinal uses (Burkill, 1985). Its common names includes air plant, Canterbury bells,Mexican love plant,resurrection plant etc. The plant is native to Madagascar and southern Africa (Biosecurity Queensland, 2011).

It is a perennial herb growing widely and used in folkloric medicine in tropical Africa, India, China, Australia and tropical America (Engler, 1926; Balzer, 1949). A number of active compounds, including flavonoids, glycosides, steroids, bufadienolides and organic acids, have been identified in *Bryophyllum pinnatum* (Costa *et al.*, 1995; Gaind and Gupta, 1972, 1974; Marriage and Wilson, 1971). Kirtikar and Basu (2000) reported that the leaf of the plant is rich in alkaloids, triterpenes, glycosides, flavanoids, steroids and lipids.

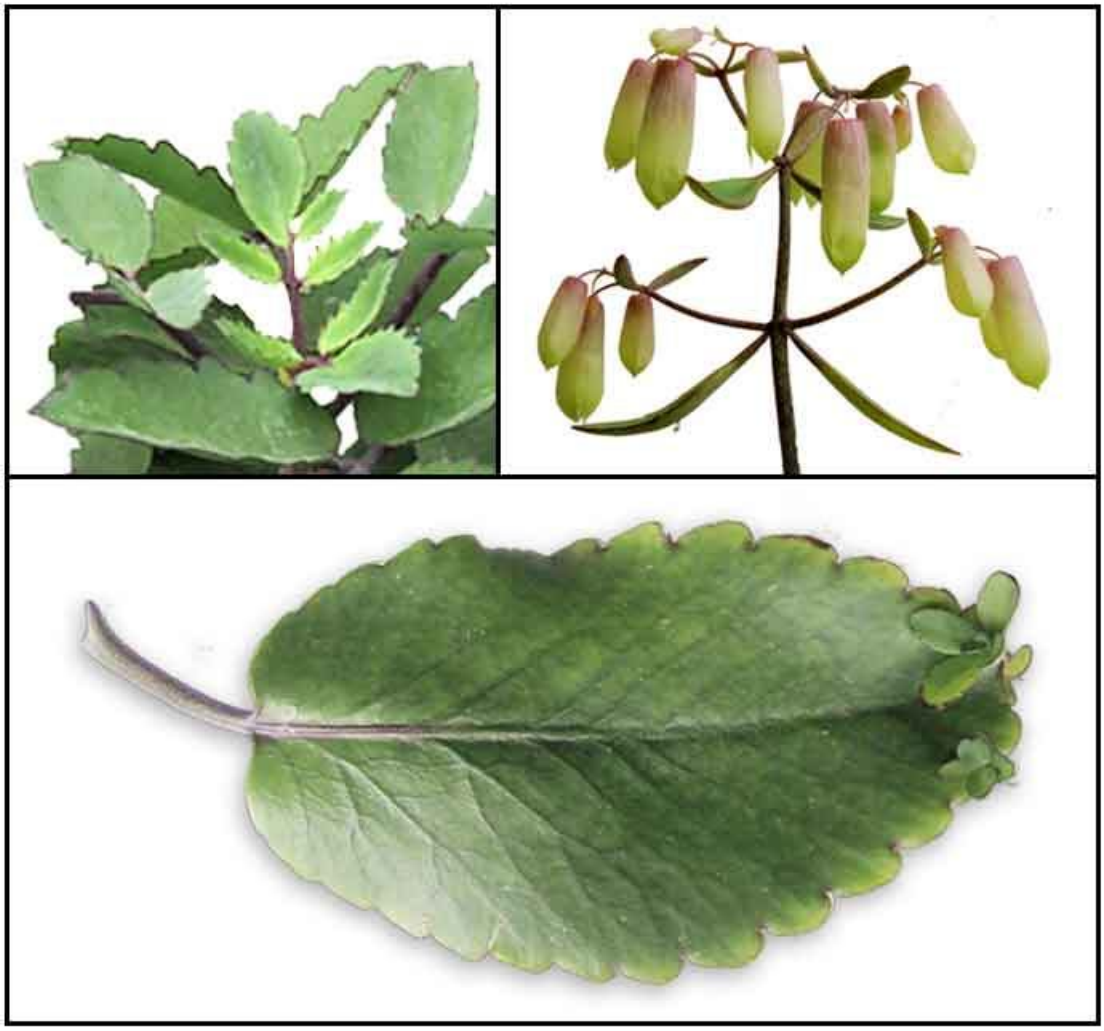

Figure1.0: Picture of the leaves and flowers of *Bryophyllum pinnatum* (Biosecurity Queensland, 2011)

It is a succulent glabrous herb, stems obtusely 4-angled, the older light coloured, the younger reddish speckled white, leaves variable, decussate, the lower usually simple or occasionally compound, the upper usually 3-5 or 7-folliolate, long petiole, petiole united by a ridge round the stem. Leaflet ovate or elliptical, crenate or serrate (Kirtikar and Basu, 2000).

It is called “ewe abamoda or odundun” by the Yoruba tribe of Southwestern Nigeria, “odaa opue” among the Igbos. It is referred to as “da bu si” in Chinese (Ghasi *et al.*, 2011; Iwu, 1993) and “sutura” by the Hausa peoples of Nigeria

1.4 Reproductive Biology

B. pinnatum is an erect, succulent, perennial shrub that grows about 1.5 m height and reproduced from seeds and also vegetatively from leaf bubbils (Agoha, 1974). It is an introduced ornamental plant that is now growing as weed around plantation crops (Dalziel, 1955). Its fleshy (i.e. succulent) leaves are capable of taking root and developing into new plants after being broken off the main plant or being dumped in garden waste (Biosecurity Queensland, 2011).

1.5 Uses of *Bryophyllum pinnatum*

The leaves of *Bryophyllum pinnatum* have been reported to possess antimicrobial (Mehta and Bhat, 1952; Akinpelu, 2000; Oliver-Bever, 1983), antifungal (Misra and Dixit, 1979), anti ulcer (Pal and Nag, 1991), anti-inflammatory and analgesic (Pal and Nag, 1989; 1992), and antihypertensive (Ojewole, 2002) activities. The methanol extract of the leaf of the plant has also been reported to have histamine receptor (H_1) antagonism in the ileum, peripheral vasculature and bronchial muscle (Pal *et al.*, 1999).

Bryophyllum pinnatum is used in ethnomedicine for the treatment of earache, burns, abscesses, ulcers, insect bites, diarrhea and lithiasis (Chopra *et al.*, 1956; Agoha, 1974; Ofokansi *et al*., 2005). In Southeastern Nigeria, this herb is used to facilitate the dropping of the umbilical cord of new borns (Dalziel, 1955). The plant leaf is mildly exposed to heat and the juice extracted and applied to the baby’s umbilical cord on daily basis. The crushed leaves as well as the extracted juice are mixed with shear butter or palm oil and rubbed on abscesses or other swellings. The leaves of *Bryophyllum pinnatum* have been reported to possess

antimicrobial (Akinpelu, 2000; Oliver-Bever, 1983; Mehta, 1952), antifungal (Misra and Dixit, 1979), anti ulcer (Pal and Nag, 1991), anti-inflammatory and analgesic (Pal and Nag, 1989; 1992), and antihypertensive (Ojewole, 2002) activities. The methanol extract of the leaf of the plant has also been reported to have histamine receptor (H_1) antagonism in the ileum, peripheral vasculature and bronchial muscle (Pal *et al*., 1999).

In Sierra Leone, a cough medicine is made from the roots while in Ivory Coast, the root sap is taken for attacks of epilepsy (Iwu, 1993). In Ghana, the leaves of the plant are rubbed on the head and inserted in the nostrils to relieve headache (Iwu, 1993). Curative and analgesic actions are also claimed for the plant (Iwu, 1993). It has been reported that the leaves are taken in West India for the treatment of arthritis and to clean the bladder (Seaforth *et al*, 1983). The plant is listed by Wong (1976) for the treatment of earache, sprains and dysmenorrhoea. Topical application of the plant extract is used for the treatment of external ulcers and burns (Wong, 1976). Among the Igbos of Nigeria, *Bryophyllum pinnatum* is believed to have antihypertensive activity. Decoctions of the leaf are usually taken to lower the blood pressure. Some people also chew the raw leaves of the plant for this purpose (Ghasi *et al*, 2011).

1.6 Statement of the Research Problems

One important area in which herbal medicines enjoy high patronage worldwide is in the management of nervous system disorders. A good number of natural product scientists believe that the initial selection of plants with diverse application in traditional medicine might be encouraged by their easily noticeable CNS effects (Amos *et al*., 2001).

Although, studies have shown the relative important effect of some medicinal plant on the central nervous system activities (Dorr *et al*., 1971; Fujimori, 1995; Wakeel, 2004).

Bryophyllum pinnatum has been used since 1921 in traditional medicine as an antipsychotic agent (Salahdeen and Yemitan, 2006).

The growing interest in herbal medicine currently witnessed globally in most circles is viewed as a welcome development. In many African countries today, a place of prominence is being given to herbal medicine both for curative and nutritional purposes (Adewunmi and Ojewole, 2004).

Therefore a close scientific study of many such plants used locally in traditional medicine is of paramount importance.

1.7 Justification of the Study

Natives of Northern Nigeria use the leaves of this plant to treat pain and inflammation and other CNS disorders.

There is also paucity of information on motor coordination deficit, anti-convulsing activities as well as the sedative ability of *Bryophyllum pinnatum* in the available literatures, hence the need to investigate the above-mentioned parameters.

1.8 General Aim of the Study

The aim of the study was to determine the neurobehavioural activities of *Bryophyllum pinnatum.*

1.9 Specific Objectives of the Study

The objectives of the present study were to:

1. Evaluate the effects of the extract of *Bryophyllum pinnatum* on motor cordination in mice.

2. Evaluate the exploratory behaviour in mice upon administration of the plant extract of *Bryophyllum pinnatum* in mice.

3. Investigate the sleeping time of the extract of *Bryophyllum pinnatum* on diazepam-induced sleep in mice.

4. Investigate the anti-convulsive effect of the extract of *Bryophyllum pinnatum* on pentylenetetrazol (PTZ)-induced convulsion in mice.

1.10 Statement of Research Hypotheses

The null (Ho) hypotheses upon which this research was based on is that the leaves of *Bryophyllum pinnatum* does not possess any central nervous system depressant or sedative ability.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Sleep in neurobehaviour

2.1.1 Definition of sleep

Guyton and Hall (2006), defined sleep as unconsciousness from which the person can be aroused by sensory or other stimuli. It is a reversible, physiological state with reduced motility and reduced responsiveness to sensory stimuli (Stenbreg, 2007).

Sleep is a naturally recurring state characterized by reduced or absent consciousness, relatively suspended sensory activity, and inactivity of nearly all voluntary muscles. It is distinguished from quiet wakefulness by a decreased ability to react to stimuli, and is more easily reversible than being in hibernation or a coma. Sleep is also a heightened anabolic state, accentuating the growth and rejuvenation of the immune, nervous, skeletal and muscular systems. It is observed in all mammals, all birds, and many reptiles, amphibians, and fish (Rosenthal, 1998).

The purposes and mechanisms of sleep are the subject of intense research. Sleep is often thought to help conserve energy, but actually decreases metabolism only about 5–10% (Prinz, 1977). Hibernating animals need to sleep despite the hypometabolism seen in hibernation, and in fact they must return from hypothermia to euthermy in order to sleep, making sleeping "energetically expensive" (Daan *et al.*, 1991).

Sleep is a very important drive. Studies performed on rats at the University of Chicago suggest that total sleep deprivation leads to death within weeks. Sleep is also an insistent drive; while people can deprive themselves of food and water until they die, they cannot voluntarily sleep deprive themselves to death. Indeed, people will drift into sleep, even if they

are in a situation (*i.e.,* driving a car) in which sleeping will mean their death (Rechtschaffen *et al.*, 1989).

It is extremely important to understand the basic phenomena of sleep. Many drugs have an affect on sleep, either directly (benzodiazepines, barbiturates, stimulants) or indirectly (opiates, melatonin). At least four of the top 10 drugs prescribed have some effects on sleep (Cardinale, 1998). It is a state of altered awareness and a condition of greatly modified electrophysiological activity which might be expected to be accompanied by profound physiological changes in the body. The brain gradually becomes less responsive to visual, auditory and other environmental stimuli during transition from wake to sleep, which is considered to be stage I of sleep (Kryger *et al.*, 2005).

2.1.2 Sleep cycle

Sleep is divided into a 90 minute cycle of NREM sleep and REM sleep. This cycle is repeated 3-6 times during the night. Generally, a night of sleep begins with about 80 minutes of NREM and 10 minutes of REM sleep. As the sleep cycle progresses through the night there is less stage 3,4 NREM sleep and more REM sleep. Thus, there is more REM sleep on towards morning (Rosenthal, 1998).

Sleep is subdivided into REM sleep (rapid eye movement sleep, paradoxical sleep, active sleep, Deep sleep, REMS), and NREM sleep (non-REM sleep, NREMS) (Stenberg, 2007). REMS is sometimes referred to as "dream sleep," although dreams can occur at any stage in the sleep cycle. NREM sleep consists of stages 1, 2, 3 and 4. REM and NREM stages are characterized by EEG (electroencephalogram) measurements, as well as by other physiological correlates such as the EMG (electro-myogram) and the EOG (electro-oculogram) (Rosenthal, 1998). The EEG measures small changes in voltage between electrodes placed on the scalp. EEG doesn't measure the discharge of a single neuron, rather

it measures the summed postsynaptic potential of large portions of nervous tissue. Waves are measured for changes in duration (frequency) and in amplitude.

Waves are categorized as either synchronized or desynchronized. Synchronized waves are high amplitude, low frequency waves. They are seen during NREM sleep and represent a coactivation of a large number of neurons. Desynchronized waves are seen when the brain is very active (*i.e.,* during wakefulness and REM sleep) and are low amplitude, high frequency waves. In addition to the electroencephalogram, EMG and EOG are often used in sleep laboratories to give information about the sleep cycle. EOG measures eye movements. During REM sleep, the rapid eye movements that give REM sleep its name is observed. EMG measures muscle activity. It is highest during wakefulness, lower during NREM sleep, and, except for occasional twitches, flat during REM sleep, as muscle atonia occurs during REM sleep (Rosenthal, 1998).

Muscle atonia is a characteristic of REM sleep that has been utilized by sleep researchers in order to preferentially deprive lab animals of REM sleep. The "flowerpot" technique involves placing an animal (*i.e.,* a rat) on top of an upside down flower pot in a bucket of water. The rat has ample room when it is awake, and can enter NREM sleep, but as soon as it enters REM sleep, it loses muscle tone, falls off the flowerpot, and splashes down into the bucket of water. It soggily and grumpily climbs back up onto his flowerpot, and goes back into NREM sleep. During the next entry into REM sleep, he's back in the water. Thus it is preferentially deprived of REM sleep (Rosenthal, 1998).

2.1.3 Wakefulness

There are two main types of EEG waves during wakefulness. Beta waves are desynchronized; low voltage (low amplitude), and of a high-mixed frequency. They are seen when the patient is alert and engaged. Alpha waves are low voltage, mixed frequency waves, and are more

synchronous than beta waves. They are seen when then subject is relaxed or drowsy, with their eyes closed (Rosenthal, 1998).

2.1.4 NREM Sleep

According to the 2007 American Academy of Sleep Medicine (AASM) standards, NREM consists of three stages and that there is relatively little dreaming in NREM (Schulz, 2008).

Stage 1 refers to the transition of the brain from alpha waves having a frequency of 8–13 Hz (common in the awake state) to theta waves having a frequency of 4–7 Hz. During this stage, the EEG shows alpha activity. This stage is sometimes referred to as somnolence or drowsy sleep. Sudden twitches and hypnic jerks, also known as positive myoclonus, may be associated with the onset of sleep during this stage. Some people may also experience hypnagogic hallucinations during this stage. During this stage, the subject loses some muscle tone and most conscious awareness of the external environment (Schulz, 2008).

Stage 2 is characterized by sleep spindles which are slow, sinusoidal waves with bursts of high amplitude peaks ranging from 11 to 16 Hz (most commonly 12–14 Hz) and K-complexes. During stage 2 sleep, which accounts for 45-55 percent of total sleep time in adults, the EEG shows low-voltage activity. The frequency is mixed, but slowing. Hypnotic agents have been shown to increase the density of sleep spindles (Rosenthal, 1998). During this stage, muscular activity as measured by EMG decreases, and conscious awareness of the external environment disappears (Schulz, 2008).

Stage 3,4 sleep, which is sometimes called Slow Wave Sleep, accounts for 15 percent of total sleep time. During Slow Wave Sleep, the EEG is synchronized. At this stage the waves are called Delta waves; they are of a high amplitude and low frequency. This is the stage in which parasomnias such as night terrors, nocturnal enuresis, sleepwalking, and somniloquy occur. Many illustrations and descriptions still show a stage 3 with 20–50% delta waves and a

stage 4 with greater than 50% delta waves; these have been combined as stage 3 (Schulz, 2008).

As sleep progressess through stages 1-4, the sleep gets "deeper" (*i.e.,* exhibiting a higher arousal threshold) and the waves become more synchronized. Stage 1 sleep is a very light stage of sleep with a low arousal threshold. It generally lasts for less than 10 minutes, at sleep onset (Schulz, 2008).

2.1.5 REM Sleep

REM sleep is usually not subdivided into stages; however, "tonic" and "phasic" aspects of REM sleep are often distinguished.

Phasic REM sleep events are intermittent (e.g., rapid eye movements and muscle twitches). Tonic REM sleep events are persistent (e.g., desynchronized [activated EEG] and striated [voluntary] muscle inhibition). This tonic versus phasic distinctions may be relevant to physiological changes that accompany sleep.

REM sleep generally makes up 20-25 percent of total sleep time in adults. In infants, REM sleep (or active sleep, as it is called in newborns) may account for up to 50 percent of sleep. Since total sleep time decreases in the elderly, the total amount of REM sleep may also fall. The absolute amount of REM sleep has been correlated with intellectual functioning in the elderly; REM sleep levels are shown to be diminished in Alzheimer's patients. REM sleep consists of tonic characteristics and phasic characteristics. Tonic characteristics are persistent throughout the entire REM sleep period, while phasic characteristics appear intermittently during the REM sleep period (Prinz *et al.*, 1982).

Tonic characteristics include a desynchronized EEG, muscle atonia and a lack of thermoregulation. Phasic characteristics include rapid eye movements, clitoral and penile

tumescence and dreams. During REM sleep, the EEG is desynchronized (low voltage, high-mixed frequency), as it is during wakefulness. Muscle atonia is a characteristic of REM sleep. Except for random twitches, skeletal muscles (except for the ocular muscles and the diaphragm) are completely relaxed. This is due to postsynaptic inhibition of motor neurons and membrane hyperpolarization. In subjects with pontine damage, REM without atonia can occur. These patients physically enact the events of their dreams. Therefore, sleep walking and sleep talking are actually NREM sleep phenomenon, since muscle tone goes flat during REM sleep (Mahowald and Schenk, 1989).

During REM sleep cells in the preoptic/anterior hypothalamus which control thermoregulation cease firing, making us essentially poikilothermic creatures during REM sleep (Parmeggiani and Rabini, 1970). Since our thermoregulatory cells stop firing during REM sleep, our body temperatures tend to approach ambient temperature. Similarly, at extremes in ambient temperature, REM sleep is lost. Animals kept at ambient temperatures in the thermoneutral zone, "that range of ambient temperatures at which the basal rate of heat production equals the rate of heat loss to the environment" show maximal levels of REM sleep (Szymusiak and Satinoff, 1981).

One of the best known phasic characteristics of REM sleep is the rapid eye movements for which the sleep state is named. Eye movements usually do not follow the action of the dream, except in some situations *(i.e.,* dreaming of a tennis match). PGO waves are a phasic burst of electrical activity between pons, lateral geniculate nucleus of the thalamus and the occipital cortex. Their exact role is unknown, although they may have a pacemaker role in the other phasic actions of REM sleep, such as rapid eye movements. They begin during NREM and may herald the onset of REM sleep. Clitoral and penile tumescence occur during REM sleep. Most REM sleep occurs towards morning; this helps to explain the erections which accompany awakening in the morning. The erections that occur during REM sleep are not

necessarily related to dream content, they are just a physiological correlate of the sleep state. It does provide a useful tool for assessing impotence in males. Since erections occur involuntarily during REM sleep, it is possible to assess whether impotence is physiological or psychological. Subjects may go to a sleep lab (or may monitor themselves at home) and monitor nocturnal penile tumescence (Rosenthal, 1998).

Finally, while dreams are what people most often associate with REM sleep, a person is not always dreaming while they are in REM sleep; dreams occupy approximately 80-90 percent of REM sleep. Also, not all dreams are REM sleep dreams. Studies from sleep labs have shown that dreaming can occur during wakefulness and NREM sleep, as well as during REM sleep. Generally, REM dreams are longer, more visual, more bizarre, and not as related to actual life events. NREM dreams tend to be shorter, more thoughtful, less emotional and more related to life events. Also, nightmares and night terrors occur during NREM sleep (Rosenthal, 1998).

2.1.6 CNS nuclei and structures involved in sleep and wakefulness

Evidence for posterior hypothalamic involvement in waking and anterior hypothalamic involvement in sleep mechanisms came from studies of patients with encephalitis in the influenza pandemic of 1917– 1919. Patients with sleep-like coma had neuronal loss in the posterior hypothalamus and rostral mesencephalic area, whereas patients with insomnia had lesions in the anterior hypothalamic area (von Economo, 1923; von Economo, 1930). Lesions in the rostral hypothalamus produced sleeplessness in rats, while waking capacity was impaired by lesions in the lateral hypothalamus, indicating the presence of an active sleeping center in the basal forebrain (Nauta, 1946). Further studies have strengthened the notion of active sleep induction by neurons in the hypothalamic preoptic area (McGinty and Szymusiak, 2001). Cats transected in the midbrain had constant sleep EEG (Bremer, 1935), indicating that preventing sensory input impaired waking. The finding that electrical

stimulation of a large mesencephalic region induced waking in cats, whereas its lesion caused sleep, led to the concept of an ascending reticular activating system (ARAS), activated by sensory input (Moruzzi and Magoun, 1949).

Lesion studies showed that REMS was generated in the pons (Jones, 1991), and specific lesions could result in REMS without muscle atonia, when the animals acted out their dream behavior (Morrison, 1993), a model of the REM Behavior Disorder of humans. Since these classical studies, several wake-promoting areas have been characterized in the posterior and lateral hypothalamus, in the tegmentum and pons, while sleep-promoting neuron groups were found to be concentrated in the hypothalamic preoptic area (Jones, 2005).

I. Basal forebrain and anterior hypothalamic neuronal groups involved in waking and sleep

The basal forebrain (BF) implied in wake/sleep regulation is a heterogeneous region adjacent to preoptic and supraoptic levels of the hypothalamus. It includes the cholinergic cell groups Ch1–4 as defined by Mesulam *et al.*, 1983, but also glutamatergic and GABAergic neurons. The cholinergic neurons are located in several nuclei, from rostral to caudal: the medial septum, the vertical and horizontal diagonal bands of Broca, the magnocellular preoptic area, the substantia innominata and the nucleus basalis of Meynert (Culliman, 1991). These neurons receive input from brainstem arousal systems, and they project widely to the cortex and limbic system without thalamic relay (Jones, 2004; Steriade and McCarley, 2005). When recorded in head-restrained rats, neurons that were immunohistochemically identified as cholinergic cells discharged in bursts during waking and REMS, and fell silent during NREMS (Lee *et al.*, 2005).

However, of BF neurons projecting to the cortex, more are GABAergic than cholinergic in the rat (Gritti *et al.*, 1997). Of those projecting to the wake-regulating lateral hypothalamus, half can be considered GABAergic, about 25% glutamatergic and only 10% cholinergic. In

addition to the majority of wake-active and wake+REMS-active neurons, some REMS-active and a few NREMS-active neurons were found in head-restrained rats, and neurons could also vary their firing patterns (Henny and Jones, 2006). Recording in freely moving rats showed similar distribution of the state dependency of neurons, and almost half of the neurons were inhibited during 3 h of sleep deprivation (SD), most of these being of wake-active type (Kostin *et al.*, 2006). Possibly, extended waking leads to inhibition of the wake-promoting BF neurons. Neurons which express Fos during sleep, and which colocalize GABA and galanin, have been found in the ventrolateral preoptic nucleus (VLPO) (Sherin *et al.*, 1998). These neurons project to waking centers like the tuberomamillary nucleus (TM) in the posterior hypothalamus, the locus coeruleus and the raphe nuclei (Sherin *et al.*,1998; Steininger *et al.*, 2001).

II. **Posterior and lateral hypothalamus**

The tuberomamillary nucleus (TM) in the posterior hypothalamus contains the only histaminergic neurons of the brain (Panula *et al.*, 1984; Haas and Panula, 2003). They receive afferent inputs from the cholinergic basal forebrain, hypothalamus and the VLPO. They project diffusely to all brain regions. Neurons in the TM fire in a robust, clocklike rhythm in waking, more slowly in NREMS and are virtually silent in REMS, and as these neurons are also inhibited by histamine auto-receptor (H3) agonists, they are presumably histaminergic (Vanni-Mercier *et al.*, 2003).

Neurons scattered in the lateral and mediolateral hypothalamus contain a peptide known as either hypocretin (Hcrt) (de Lecea *et al.*, 1998) or orexin (OX) (Sakurai *et al.*, 1998). The peptide is coded by one gene, but expressed as two forms: Hcrt1/OX-A and Hcrt2/OX-B. Hcrt/OX neurons project diffusely in the brain, innervating major waking-promoting systems (Siegel, 2004; Saper *et al.*, 2005; Jones, 2005). Inputs are from hypothalamic regions, brainstem, preoptic area, BF and amygdala (Sakurai *et al.*, 2005; Yoshida *et al.*, 2006).

Identified orexinergic neurons in head-restrained rats discharged most intensely in aroused waking, and least in REMS (Lee *et al.*, 2005).

III. **Tegmentopontine nuclei**

The tegmentopontine cholinergic nuclei are the laterodorsal tegmental nucleus (LDT, Ch6) and the pedunculopontine nucleus (PPT, Ch5) (Mesulam *et al.*, 1983; Woolf, 1991). LDT is situated medially, between the raphe and the locus coeruleus, whereas PPT is more lateral. The neurons receive input from ascending sensory fibers as well as from neighboring nuclei. Major efferent projections are to the thalamus, hypothalamus, BF, pontine reticular formation, locus coeruleus and raphe nuclei (Woolf, 1991; Jones and Cuello, 1993; losier and Semba, 1993). Some GABAergic neurons project from the LDT-PPT to the posterior lateral hypothalamus (Ford *et al.*, 1995). The discharge activity of most Ch5 and Ch6 neurons is high during wakefulness and REMS, and correlates with EEG signs of cortical activation (Steriade *et al.*, 1990; Datta and Siwek, 2002).

Cell groups in the pons and medulla named A1–A7 (Dahlstrom and Fuxe, 1964) contain noradrenaline, and the most important for sleep-wake regulation is the locus coeruleus (LC). LC and A4 project to the thalamus, neocortex, hippocampus and cerebellum, whereas the hypothalamus receives noradrenergic innervation from A1,A2,A5 and A7 (Moore and Bloom, 1979). LC neurons receive input which is partly glutamatergic and GABAergic from adjacent areas in the pons. LC neurons fire most intensely in waking, less in NREMS and least in REMS (Chu and Bloom, 1976) and changes in their firing frequency precede the change in vigilance state (Aston-Jones and Bloom, 1981). Serotonergic neurons are found in the raphe nuclei in the brainstem midline. Rostral nuclei (B4–B9) project to the forebrain, while caudal nuclei (B1–B3) project to the spinal cord (Dahlstrom and Fuxe, 1964). The dorsal raphe (DR) nucleus (B7) is important for sleep-wake regulation.

Like the LC, it receives input from adjacent tegmentopontine areas, and projects widely to the forebrain and limbic system. DR neurons also fire maximally in waking, less in NREMS and are virtually silent in REMS (McGinty and Harper, 1976). During waking, serotonergic neurons are activated during certain behaviours, like grooming and rhythmic movement, and might serve to limit cortical activation by inhibitory influences on other arousal-promoting neurons (Jones, 2005).

Dopaminergic neurons are found in the substantia nigra (A9), the ventral tegmental area (VTA, A10) and hypothalamic cell groups (A12–A14) (Dahlstrom and Fuxe, 1964). A9 projects to the striatum, and is involved in waking motor activity. A10 projects to the prefrontal, orbital and cingulate cortices, as well as to the amygdala, the nucleus accumbens and other limbic areas (Oades and Halliday 1987). A12–A14 regulate neuroendocrine functions. The activity of presumably dopaminergic neurons in A9 and A10 is not significantly modulated by the sleep-wake cycle (Trulson and Preussler, 1984; Trulson, 1985), but they increase their firing in relation to positively rewarding stimuli (Jones, 2005).

IV. **Thalamus**

The thalamus is the gate for sensory input to the cortex, and so is of paramount importance for vigilance. The excitability of thalamocortical (TC) projection neurons is modulated by innervation from the thalamic reticular nuclear complex (RE), the neurons of which are in turn modulated by input from the LDT/PPT, LC and raphe (Steriade and McCarley, 2005; Steriade, 2005). The thalamocortical network is quite complicated. TC neurons are glutamatergic and convey information to the cortex. The thalamic reticular nuclear complex (RE) neurons are GABAergic and inhibit TC neurons. They also influence each other through reciprocal connections. RE neurons are excited primarily by glutamatergic output from the cortex, but also modulated by the aminergic and cholinergic tegmentopontine nuclei. During NREMS, TC neurons are inhibited and incoming sensory messages are blocked from

reaching the cortex. Three major types of thalamocortical oscillation occur during NREMS: sleep spindles, delta activity and slow oscillations.

Activity bursts can be generated in the isolated RE nucleus when its neurons are hyperpolarized by Gprotein- coupled potassium channel activation. In situ, these bursts cause rhythmic inhibition of TC neurons, which in turn fire in bursts onto cortical neurons to yield sleep spindle activity of 12 –14 Hz frequency.

Delta waves (at 1– 4 Hz), on the other hand, rely on an intrinsic property of TC neurons to burst rhythmically when hyperpolarized to more than _65 to _70 mV. Cortical neurons also have intrinsic capability of delta frequency burst firing. The slow oscillation (<1 Hz) depends mostly on cortical neurons, and is then conveyed to thalamic RE and TC neurons through glutamatergic output. Considerable evidence shows that sleep facilitates memory consolidation (Siegel, 2004). In fact, cortical responsiveness is maintained during NREMS although sensory input is shut out, and plastic changes in neuronal networks have been found to be enabled during sleep (Steriade and Timofeev, 2003).

2.1.7 The Neurochemistry of NREM Sleep

The anterior hypothalamus and adjacent forebrain areas are important in producing NREM sleep, as are the thalamus, dorsal raphe, and nucleus tractus solitarius. Neurotransmitters such as serotonin and GABA, are located in these brain regions and play an important role in sleep. Serotonin (5-HT), found in raphe neurons of the brainstem, may be involved in sleep onset. Insomnia occurs when serotonergic cells of the dorsal raphe are lesioned. MAO Inhibitors (specific for 5-HT) enhance sleep. Also, there is evidence that substances in the biosynthetic pathway of serotonin (such as tryptophan and vitamin B6) may facilitate sleep (Jones, 1989). However, in spite of all the evidence supporting the role of serotonin in sleep

onset, there are studies which suggest that the role of serotonin in sleep is not as clear cut as it may seem. The exact role of serotonin in sleep has not yet been elucidated (Rosenthal, 1998).

The inhibitory neurotransmitter GABA is released in highest concentrations during NREM sleep. GABAergic neurons are located throughout the brain, including the basal forebrain, hypothalamus, thalamus, brainstem and cortex. Hypnotics, such as benzodiazepines (diazepam or triazolam) and barbiturates (phenobarbital, secobarbital) tend to work by potentiating GABA mediated inhibitory processes. Both bind to sites on GABA receptor complex, increase C1- flow through ionophore, causing hyperpolarizations and reduced cell firing. They may shut off neurons in the reticular activating system and inhibit transmission and activity of neurons that project to the cortex and thalamus (Rosenthal, 1998).

Overall, hypnotics do increase total sleep time, decrease sleep latency and decrease the number of awakenings. They also decrease the amount of time spent in NREM sleep stages 3,4 and, in some cases, REM sleep, which are sleep stages necessary for a normal, restorative night of sleep. Tolerance to these drugs develops fairly quickly (within two weeks) and insomnia and REM rebound are seen upon withdrawal from these drugs. Caffeine blocks adenosine receptors; it is thought that adenosine may promote sleep. Intracerebroventricular infusion of adenosine will increase total sleep time in rats (Gaillard *et al.*, 1989). Also, it is thought that benzodiazepines may in part exert their actions by blocking the reuptake of adenosine.

Monnier *et al.*, (1977) discovered that there are sleep inducing factors, which, when injected into the blood or cerebrospinal fluid of an animal, induce sleep. However, there is probably not one single blood borne element that induces sleep, as conjoined twins do not fall asleep at the same time. Many other factors are also involved to varying degrees in sleep. Opiates,

Somatostatin, Melanocyte Stimulating Hormone, Insulin, CCK, Prostaglandins, Growth Hormone and Prolactin may all play a role in sleep (Rosenthal, 1998).

2.1.8 Neurochemistry of REM Sleep

Acetylcholine is located within neurons in the pontine tegmentum and is involved with REM sleep generation. "REM On cells" are cholinergic cells in the lateral pontine and medial medullary reticular areas that innervate the thalamus, hippocampus and hypothalamus. These cells discharge at high rates during REM and show little or no activity during NREM. Physostigmine, which inhibits catabolic enzymes, precipitates the appearance of REM sleep during NREM. The injection of carbachol, a muscarinic agonist, into the pontine tegmentum induces REM sleep. Blocking muscarinic receptors will retard the appearance of REM sleep.

Two symptoms associated with narcolepsy are cataplexy and hypnagogic hallucinations, whereby the patient loses all muscle tone and falls into a dream state while laying awake and paralyzed. Some have theorized that it is the sudden appearance of REM sleep into the waking state that causes these symptoms. Indeed, narcoleptic dogs are found to have increased levels of cholinergic receptors in their pons and medial medulla (Guilleminault, 1989). Administration of cholinergic antagonists blocks these effects, which further illustrates the role of acetylcholine in REM sleep. "REM Off cells" are noradrenergic and serotonergic cells found in the locus coeruleus and raphe. These are cells which are slow or silent during REM sleep. Affecting levels of norepinephirne or serotonin can have an effect on REM sleep.

In general, antidepressants have the effect of decreasing REM sleep, which is elevated in human endogenous depression (Benca *et al.*, 1991). Generally, the effectiveness of antidepressant drugs is proportional to the degree to which the drugs diminish REM sleep (Vogel *et al.*, 1975). Tricyclic antidepressants, such as amitryptyline and imipramine, block the reuptake of norepinephrine and serotonin, as well as having an anticholinergic effect.

These drugs vary in their effectiveness as sedatives. The new generation antidepressants (*i.e.* Fluoxetine, Sertraline) act specifically to block serotonin reuptake. Most of these drugs diminish REM sleep (Rosenthal, 1998).

2.1.9 Circadian Rhythm and sleep

A circadian rhythm is an approximate daily periodicity, a roughly-24-hour cycle in the biochemical, physiological or behavioural processes of living beings (Saper *et al.*, 2005). In strict sense, circadian rhythms are endogenously generated rhythm, although can be modulated by external cues, primarily daylight. They allow organisms to anticipate and prepare for precise, and regular environment changes. Circadian rhythm is cell-autonomous at the same time, different cells may communicate with each other resulting in a synchronised output of electrical signalling. These may interfere with endocrine glands or the brain to result in periodic release of hormones. The receptors for these hormones may be located far across the body, and synchronise the peripheral clocks of various organs. Thus, information of the time of the day as relayed by the eyes travels to the clock in the brain, and through that, clocks in the rest of the body may be synchronised. This is how the timing of sleep for example, is co-ordinately controlled by the biological clock (Iber *et al.*, 2007).

Circadian rhythms are important in determining the sleeping and feeding patterns of all animals, including humans (Tosini *et al*., 2008).

2.1.10 Physiological changes during sleep

Specific physiological changes accompany sleep because nearly every bodily function changes to one degree or another during sleep. These changes include the following:

I. Nervous system changes

(a) Central Nervous System

Neurons in many different regions of the brain exhibit changes in activity related to the states of sleep and wakefulness. During NREM sleep, many neurons in the brain exhibit a reduced mean discharge rate. During tonic REM sleep, the mean firing rate in many regions increases compared with NREM or wakefulness. The high neuronal firing rates during REM sleep refute the old hypothesis that the brain rests during sleep. A number of regions, particularly the visual system, exhibit very great phasic increases in neuronal discharge during REM sleep. In a corresponding manner, the overall metabolism of brain tissue is generally decreased in most brain areas during NREM sleep.

(b) Autonomic Nervous System.

Because the autonomic system operates without conscious control, it is understandable that this system is very relevant to the sleeping organism. A few generalizations serve to highlight the function of the autonomic nervous system during sleep.

During NREM sleep, sympathetic activity stays at about the same level as during relaxed wakefulness, but parasympathetic activity increases resulting in a slight predominance of parasympathetic over sympathetic drives. During tonic REM sleep, the imbalance swings even more in favor of parasympathetic activation, not because parasympathetic input increases above the NREM level, but because the level of sympathetic input declines from that present during NREM sleep. During phasic REM sleep, both sympathetic and parasympathetic input increase (like putting your feet down on the accelerator and the brake at the same time). Transient imbalances in these autonomic drives generally favour a slight preponderance of sympathetic activation (Rosenthal, 1998).

II. Cardiovascular system

During NREM, there is an overall reduction in heart rate, cardiac output and blood pressure, due to a general vasodilation. During REM sleep, there are variations in blood pressure and heart rate, but overall the rates are increased, especially during the phasic events of REM sleep, probably due to a generalized vasoconstriction seen in the skeletal muscles during phasic REM sleep.

With the transition from relaxed wakefulness to NREM sleep, systemic blood pressure tends to decrease slightly and to exhibit reduced variability. During tonic REM sleep, blood pressure stays at about the NREM level. During phasic REM sleep, on the other hand, blood pressure is highly variable and may occasionally increase as much as 40 mmHg, which is about 30% over the resting level. Cardiac output is reduced during NREM sleep versus relaxed wakefulness, and continues at about the same level during REM sleep. During NREM sleep there is active vasodilation of the vessels that supply resistance to the circulatory system. Most vessels remain dilated during tonic REM sleep, except for those of skeletal (striated) muscles, which undergo vasoconstriction. Vasoconstriction is generalized during phasic REM sleep, which may be the mechanism that results in greatly increased blood pressure during phasic REM sleep.

Studies of regional cerebral blood flow that have been carried out in cats have shown that only a few brain areas receive greater blood flow during NREM sleep than during relaxed wakefulness, although in general the increase is not very great. During tonic REM sleep, on the other hand, most brain areas show greatly increased blood flow, almost uniformly greater than 50% above the waking level, and as great as nearly 200%. During phasic REM sleep, there are transient further increases in blood flow to most brain regions, although precise quantification is difficult because the phasic episodes are so short. Brain temperature is generally decreased during NREM sleep versus wakefulness and there are occasionally

increases above the waking level during REM sleep. These changes in brain temperature are correlated with changes in the temperature of the blood supplying the brain, even though the brain is slightly warmer than the blood (by 0.2-0.6^{o}C).

One of the possible functions of sleep is to give the heart a chance to rest from the constant demands of waking life. As compared to wakefulness, during non-REM sleep there is an overall reduction in heart rate and blood pressure. During REM sleep, however, there is a more pronounced variation in cardiovascular activity, with overall increases in blood pressure and heart rate. Additionally, changes in blood flow that cause erections to occur in males or swelling of the clitoris in females is characteristic of REM sleep The underlying reason for these considerable neural and physiological variations in REM sleep may be a by-product of REM-related changes in nervous system activity or related to dream content (Pace-Schott and Hobson,2002).

III. Respiration

Different processes exist to maintain the regulation of respiration during sleep than during waking. Some cells related to breathing stop firing during Slow Wave Sleep, some in REM. Overall, there is slight hypercapnia, a decrease in total ventilation, and a decreased sensitivity to inspired CO_2. During NREM there is a slight hypoventilation. This is due to a relaxation of upper airway muscles, as well as a decrease in the firing of inspiratory neurons, which show a decreased sensitivity to stimuli. PCO_2 levels rise while PO_2 levels fall. During NREM sleep, breathing is under chemical and mechanical feedback control. During REM there is an overall higher and variable respiratory rate. It appears as though different processes maintain respiration during REM sleep, and it is not driven by vagal signals or peripheral or central chemoreceptors. It may be driven by higher cortical control, which may explain the variable rate (Rosenthal, 1998). There is a lower tidal volume, and higher respiratory rate. As REM sleep is associated with a loss of muscle tone, there is an increase in the resistance of the

upper airway. Obstructive sleep apnea can occur in certain individuals when the airway resistance increases to a degree which totally blocks ventilation. Sleep Apnea is a lack of breathing (for at least 10 seconds) during sleep. This occurs at both ends of the age spectrum, it is seen in older patients, as well as in infants (SIDS). In adults, it is associated with daytime sleepiness and snoring. These patients are generally older, overweight males. SIDS (sudden infant death syndrome) is the leading cause of post-neonatal mortality. SIDS may be genetic and related to apnea in adults. Most deaths occur early in the morning, when SIDS babies have an increase in REM sleep and fewer awakenings. So there is a possibility that these infants have an increased arousal threshold, and don't awaken to increased PCO_2. An increased incidence of SIDS is seen with younger mothers, low birth weight, cocaine use and poverty, and there is the speculation that many of the cases may be due to parental neglect or abuse. However, there has been a sharp decrease in the incidence of SIDS in the last few years, when parents began to be advised to place their children on their backs for sleeping (Rosenthal, 1998).

Breathing patterns also change during sleep. During wakefulness, breathing is usually quite irregular, since it is affected by speech, emotions, exercise, posture, and other factors. As we progress from wakefulness through the stages of non-REM sleep, our breathing rate slightly decreases and becomes very regular. During REM sleep, the pattern becomes much more variable again, with an overall increase in breathing rate (Pace-Schott and Hobson, 2002).

IV. Kidneys

Many sleep-related changes affect kidney function. During sleep, urine production decreases, and the concentration of urine increases. There is a decrease in the glomerular filtration rate and the renal plasma flow. Secretion of aldosterone increases, as does ADH, both of which contribute to the decreased production of urine. There is also an increase in prolactin secretion, which some consider to potentiate the action of aldosterone. There is increased

parathyroid hormone release during sleep, which may affect calcium excretion. In general, the following are reduced during sleep: glomerular filtration rate, renal plasma flow, filtration fraction, and the excretion of sodium, chloride, potassium, and calcium. Smaller quantities of more concentrated urine are excreted during NREM sleep than during wakefulness; during REM sleep urine excretion is reduced and concentrated to a greater extent than during NREM sleep.

V. Digestive system

A number of interesting relationships between sleep and the digestive system have been reported. The motility of the GI tract decreases during sleep in individuals with a normal digestive system. Gastric acid secretion also decreases during sleep, except in patients with duodenal ulcers, who show an increase of gastric acid secretion of 3-20 times normal levels during sleep. The swallowing reflex normally slows down during sleep, which helps explain the little puddle of drool left on your pillow.

VI. Endocrinology

Stage 3,4 sleep is associated with increased secretion of Growth Hormone, especially in children approaching puberty. Prolactin secretion is also tied to sleep; its secretion rises about 30-90 minutes after the onset of sleep. Sleep onset inhibits the release of cortisol. Although it has become a very popular treatment for insomnia, the secretion of melatonin does not appear to be related to sleep cycles. Melatonin, which is synthesized in the pineal gland, is released at night, and is inhibited by light. While exogenous administration increases total sleep time and decreases sleep latency, its exact role in sleep is yet to be elucidated. At this point, there is no evidence that endogenous melatonin release is involved in sleep processes; it is thought that it may affect sleep by affecting circadian pacemaker or other factors *(i.e.,* body temperature) which indirectly affect sleep (Rosenthal, 1998).

VII. Thermoregulation

Through a process known as thermoregulation, the temperature of our body is controlled by mechanisms such as shivering, sweating, and changing blood flow to the skin, so that body temperature fluctuates minimally around a set level during wakefulness. Just before we fall asleep, our bodies begin to lose some heat to the environment, which some researchers believe actually helps to induce sleep. During sleep, our central set temperature is reduced by 1 to 2°F. As a result, we use less energy maintaining our body temperature. It has been hypothesized that one of the primary functions of sleep is to conserve energy in this way. Body temperature is still maintained, although at a slightly reduced level during non-REM sleep, but during REM sleep our body temperature falls to its lowest point. Curling up in bed under a blanket during the usual 10- to 30-minute periods of REM sleep ensures that we do not lose too much heat to the environment during this potentially dangerous time without thermoregulation (Hobson and Pace-Schott, 2002).

At sleep onset, the body temperature set point is lowered and body temperature falls. The body therefore employs heat loss mechanisms (sweating) to cool down the body to the new set point. Thermoregulatory cells in the preoptic/anterior hypothalamus slow firing during NREM sleep, only to stop firing entirely during REM sleep, when thermoregulation ceases (Rosenthal, 1998).

VIII. Reproduction

Activation of the sexual organs during sleep is a commonplace occurrence in both males and females. In women, vaginal blood flow has been measured with a thermoconductance flow meter. In men, the most common measurement technique involves placing two small elastic circular mercury-filled capillary strain gauges around the penis, one at the base and one at the tip. The strain gauges measure the changes in penile diameter that occur with tumescence. Both types of measures are recorded on a polysomnograph along with EEG, EOG, and EMG

activity. By this means changes in vaginal blood flow or in penile circumference can be correlated with the different stages of sleep and wakefulness (Rosenthal, 1998).

Studies in normal males from 3 to 79 years of age have shown that REM sleep-related penile tumescence occurs in all normal, healthy males. Episodes of tumescence are clearly associated with REM sleep periods, although tumescence is not exclusively confined to REM sleep, particularly in adolescents. The peak period of tumescence time as a percentage of REM sleep time occurs in the mid-teens. The few studies that have been performed in females have found a similar relationship between sexual arousal and REM sleep, although the link between sexual arousal and REM sleep does not appear to be as strong as in males. Among the earliest studies of penile tumescence during sleep were those attempting to relate tumescence to dream content. However, tumescence was found to occur in conjunction with REM sleep whether or not the dream content was sexual in nature (Rosenthal, 1998).

There is some evidence that the occurrence of ejaculation during REM sleep (wet dreams) is more likely to occur if there is sexual content in the accompanying dream. In dreams in which anxiety, aggression, or rejection are prominent emotional phenomena, the accompanying tumescence shows very small, short-lived reductions in circumference.

The most common use of tests for nocturnal penile tumescence is to differentiate between organic impotence and impotence that is of psychogenic origin. For example, a male who is unable to have an erection during wakefulness for psychological reasons will have normal periods of tumescence during REM sleep. If tumescent episodes are not present during REM sleep, one would conclude that the impotence was of an organic nature. It should also be pointed out that few diagnostic tools are entirely accurate, for there are some recent data that nocturnal penile tumescence may also decrease in depression (Rosenthal, 1998).

IX. Infection

During systemic infections, people often experience increased lassitude or sleepiness. Unfortunately, studies of the relationship of infection to sleep have not been performed in humans. The sleepiness associated with hepatic failure may be relieved in seconds by the administration of benzodiazepine receptor antagonists. In experimental animals, initial work in this area indicates that: 1) very large changes in sleep patterns occur during infection; 2) sleep changes are a major sign of infectious disease; and 3) sleep changes are adaptive and possibly play a role in nonspecific host defenses. (A contrary note is that prolonged sleep deprivation does not reduce any of several measures of immune response). The general pattern after bacterial or fungal infection is an initial period of enhanced NREM sleep, followed 1-2 days later by a period of suppressed NREM. REM sleep is inhibited throughout the course of infection. It is clear that during infection and illness there is an increased tendency to sleep; it is suspected, but not proven, that sleep facilitates the healing process (Rosenthal, 1998).

X. Hearing

During slow wave sleep, we see the highest auditory arousal threshold, especially in children. One study showed that an auditory signal of 120 dB (comparable to the sound of a jet engine form 500 feet) was inadequate to wake school aged children. This auditory arousal has been shown to depend on the stimuli. If the stimulus is significant to the subject, they are more likely to awaken. For instance, a subject is more likely to awaken to the sound of her own name than to another name, or more likely to awaken to the sound of her own baby crying, rather than to the sound of another baby (Chase, 1993).

XI. Memory processing

Turner *et al*, 2007, reported that working memory can be affected by sleep deprivation. Working memory is important because it keeps information active for further processing and supports higher-level cognitive functions such as decision making, reasoning, and episodic memory.

Memory is affected differently by certain stages of sleep such as REM and slow-wave sleep (SWS). Direct current stimulation to the prefrontal cortex increases the amount of slow oscillations during SWS. The direct current stimulation greatly enhanced word-pair retention, giving evidence that SWS plays a large role in the consolidation of episodic memories (Walker, 2009).

2.1.11 Sleep disorders

Insomnia is a symptom of difficulty in initiating and maintaining sleep or experiencing non-refreshing sleep and is associated with day time sequelae (Roth and Roehrs, 2003). It is the most common sleep-related complaint and the second most common overall complaint (after pain) reported in primary care settings (Mahowald *et al.*, 1997). It is costly and can cause significant morbidity if not addressed appropriately (Attarian, 2003). The use of benzodiazepines in the management of insomnia is associated with well known problems such as 'hangover' effects, dependence, addiction, withdrawal symptoms and subsequent drug resistance (Tyrer, 1989).

Other sleep disorders include:

a) **Bruxism**: Involuntary grinding or clenching of teeth while sleeping.
b) **Delayed sleep phase syndrome and advanced sleep phase syndrome**: Inability to awaken and fall asleep at socially accepted time.
c) **Hypnoea syndrome**: Abnormal shallow breathing while sleeping.

d) **Narcolepsy**: the condition of falling asleep spontaneously and unwillingly at inappropriate times.

e) **Night terror**: abrupt awakening from sleep with behaviour consisting with terror.

f) **Parasomnias**: This includes a variety of disruptive sleep-related events.

Others include: Periodic limb movement disorder, rapid eye movement, behaviour disorders, restless leg syndrome, obstructive sleep apnoea, sleep paralysis and sleep walking (Chokroverty, 1999).

2.1.12 Plants used in sleep management

The ethanol leaf extract of *Bryophyllum pinnatum* have been reported to cause a significant effect on sleep. It showed a marked sedative effect and potentiated sleep in pentobarbitone-induced sleeping time in mice (Lokesh *et al.,* 2009; Pal *et al.,* 1999; Salahdeen and Yemitan 2006).

Magaji *et al.,* (2007), reported that the methanolic fractions of *Securinega virosa* possess a potent sedative activity. The ethanolic root extract of *Coccos nucifera* was reported by Dilipkumar *et al.,* (2011) to significantly potentiate the duration of pentobarbital, diazepam and meprobamate-induced sleep in mice, suggesting probable tranquilizing action as well as CNS depressant action. Ezekiel *et al.,* (2010) showed that the hydro-ethanolic extract of *Commiphora africana* significantly and dose-dependently reduced the onset and prolonged the duration of sleep-induced by diazepam.

2.2 Epilepsy and *Bryophyllum pinnatum*

A **convulsion** is a medical condition where body muscles contract and relax rapidly and repeatedly, resulting in an uncontrolled shaking of the body (International League Against Epilepsy 1993). During convulsions, the person's muscles contract and relax repeatedly.

Since a convulsion is often a symptom of an epileptical seizure, the term *convulsion* is sometimes used as a synonym for *seizure*. However, not all epileptic seizures lead to convulsions, and not all convulsions are caused by epileptic seizures (Pollack, 2006).

The British Epilepsy Association in 2012 defined epilepsy as a tendency to have recurrent seizures (sometimes called fits). A seizure is caused by a sudden burst of excess electrical activity in the brain, causing a temporary disruption in the normal message passing between brain cells. This disruption results in the brain's messages becoming halted or mixed up.

Epileptic seizures result from abnormal, excessive or hypersynchronous neuronal activity in the brain (Fisher *et al.*, 2005). Epilepsy is characterized by a long term risk of recurrent seizures. These seizures may present in a number of different ways (Duncan *et al*., 2006).

Sridharan (2002), stated that epilepsy is the second most common neurological disorder attended to by neurologists. The estimated prevalence of this condition is 8 to 13 per thousand people in Nigeria (Azubuike and Nkanginieme, 1996). About 50 million people worldwide have epilepsy at any one time (WHO, 2001). Epilepsy is usually controlled, but not cured, with medication. About 30% of epilepsy is refractory to treatment (WHO, 2001).

Epilepsy is an important health problem. Around 30,000 people develop epilepsy every year and the condition will affect about one person in 20 at some time during their lives. There are around 20 to 70 new cases of epilepsy per 100,000 people per year (Shporvan, 1990).

Pal *et al.,* (1999) reported the extract of *Bryophyllum pinnatum* could not offer any protection against convulsion induced by either pentylenetetrazole or strychnine in mice (although the results indicate that the leaf extract prolonged the time of death after convulsion). Salahdeen and Yemitan (2006) also reported that the plant showed a reduction in duration of seizures in

mice treated with the aqueous leaf extract of the plant. Lokesh *et al.,*(2009) reported that the plant showed some degree of protection against Strychnine and Picrotoxin-induced seizure.

2.2.1 Types of seizures

It is very important to distinguish and understand the various types of seizures, since they may have different causes and treatments. The International League Against Epilepsy (ILAE) in 1981, proposed a classification scheme for individual seizures. This classification is based on observation (clinical and EEG).

Seizures may be organized as partial (focal or onset seizures) or generalized (distributed) according to origin within the brain (ILAE, 1993).

(a) **Partial Seizures**

Focal epilepsy can involve almost any local part of the brain, either localized regions of the cerebral cortex or deeper structures of both the cerebrum and brain stem. Most often, focal epilepsy results from some localized organic lesion or functional abnormality, such as (1) scar tissue in the brain that pulls on the adjacent neuronal tissue, (2) a tumor that compresses an area of the brain, (3) a destroyed area of brain tissue, or (4) congenitally deranged local circuitry (Guyton and Hall, 2006).

Localization-related epilepsies, sometimes termed partial or focal epilepsies, arise from an epileptic focus, a small portion of the brain that serves as the irritant driving the epileptic response. It may be further divided into Simple and complex (Psychomotor) seizures depending on the extent to which consciousness is affected. If it does not affect consciousness, it is termed a simple partial seizure and if it affects consciousness to some degrees, it is called complex partial seizure (Wilson and Reynolds, 1990).

Psychomotor seizure, which may cause (1) a short period of amnesia; (2) an attack of abnormal rage; (3) sudden anxiety, discomfort, or fear; and/or (4) a moment of incoherent speech or mumbling of some trite phrase. Sometimes the person cannot remember his or her activities during the attack, but at other times, he or she is conscious of everything that he or she is doing but unable to control it. Attacks of this type frequently involve part of the limbic portion of the brain, such as the hippocampus, the amygdala, the septum, and/or portions of the temporal cortex (Guyton and Hall, 2006). Still, other patients can perform complicated actions, such as travel or shopping, while in the midst of a complex partial seizure (Guyton and Hall, 2006; The National Society for Epilepsy, 2009).

When the effects of a partial seizure as a 'warning sign' occurrs before a larger seizure, they are known as an aura: it is frequently the case that a partial seizure will spread to other parts of the brain and eventually become generalized, resulting in a tonic-clonic convulsion. The subjective experience of an aura, like other partial seizures, will tend to reflect the function of the affected part of the brain (The National Society for Epilepsy, 2009).

(b) **Generalized Seizures**

Depending on the effects on the body, generalized seizures are further divided into absence (petit mal), myoclonic, clonic, tonic, tonic-clonic (grand mal), and atonic seizures (Simon 2004).

Absence seizures involve an interruption to consciousness where the person experiencing the seizure seems to become vacant and unresponsive for a short period of time (usually up to 30 seconds). Slight muscle twitching may occur.

Myoclonic seizures involve an extremely brief (< 0.1 second) muscle contraction and can result in jerky movements of muscles or muscle groups.

Clonic seizures are myoclonus that are regularly repeating at a rate typically of 2-3 per second.

Tonic-clonic seizures involve an initial contraction of the muscles (tonic phase) which may involve tongue biting, urinary incontinence and the absence of breathing. This is followed by rhythmic muscle contractions (colonic phase). This type of seizure is usually what is referred to when the term 'epileptic fit' is used colloquially.

Atonic seizures involve the loss of muscle tone, causing the individual to fall to the ground. These are sometimes called 'drop attacks', but should be distinguished from similar looking attacks that may occur in narcolepsy or cataplexy (Wilson and Reynolds, 1990)

(c) **Continuous seizure**

Continuous seizures includes:

i. **Status epilepticus** which refers to continuous seizure activity with no recovery between successive seizures. When the seizures are convulsive, it is a life-threatening condition, and emergency medical assistance should be called immediately if this is suspected. A tonic-clonic seizure lasting longer than 5 minutes (or two minutes longer than a given person's usual seizures) is usually considered grounds for calling the emergency services (Wilson and Reynolds, 1990).

ii. **Epilepsia partialis continua** which is a rare type of focal motor seizure (hands and face) which recurs every few seconds or minutes for extended periods (days or years). It is usually due to strokes in adults and focal cortical inflammatory processes in children (Rasmussen's encephalitis), possibly caused by chronic viral infections or autoimmune processes (The National Society for Epilepsy, 2009).

2.2.2 Diagnosis of Epilepsy

Epilepsy covers conditions with different aetiologies, natural histories and prognoses, each requiring different management strategies. A full medical diagnosis requires a definite categorization of seizure and syndrome types (Panayiotopoulos and Koutroumanidis, 2005).

Epilepsy classification includes more information about the person and the episodes than seizure type alone, such as clinical features (e.g., behavior during the seizure) and expected causes (Simon, 2004).

2.2.3 Epileptic Syndromes

There are four main groups of epileptic syndrome which can be further divided into: benign Rolandic epilepsy, frontal lobe epilepsy, infantile spasms, juvenile myoclonic epilepsy, juvenile absence epilepsy, childhood absence epilepsy (pyknolepsy), hot water epilepsy, Lennox-Gastaut syndrome, Landau-Kleffner syndrome, Dravet syndrome, progressive myoclonus epilepsies, reflex epilepsy, Rasmussen's syndrome, temporal lobe epilepsy, limbic epilepsy, status epilepticus, abdominal epilepsy, massive bilateral myoclonus, catamenial epilepsy, Jacksonian seizure disorder, Lafora disease, photosensitive epilepsy, etc.

Each type of epilepsy presents with its own unique combination of seizure type, typical age of onset, EEG findings, treatment, and prognosis. The most widespread classification of the epilepsies (ILAE, 1989), divides epilepsy syndromes by location or distribution of seizures (as revealed by the appearance of the seizures and by EEG) and by cause. Syndromes are divided into localization-related epilepsies, generalized epilepsies, or epilepsies of unknown localization.

Localization-related epilepsies, sometimes termed partial or focal epilepsies, arise from an epileptic focus, a small portion of the brain that serves as the irritant driving the epileptic response. Generalized epilepsies, in contrast, arise from many independent foci (multifocal

epilepsies) or from epileptic circuits that involve the whole brain. Epilepsies of unknown localization remain unclear as to whether they arise from a portion of the brain or from more widespread circuits (Wilson and Reynolds, 1990).

Epilepsy syndromes are further divided by presumptive cause: idiopathic, symptomatic, and cryptogenic. In general, idiopathic epilepsies are thought to arise from genetic abnormalities that lead to alteration of basic neuronal regulation (Aur, 2011). Symptomatic epilepsies arise from the effects of an epileptic lesion, whether that lesion is focal, such as a tumor, or a defect in metabolism causing widespread injury to the brain. Cryptogenic epilepsies involve a presumptive lesion that is otherwise difficult or impossible to uncover during evaluation.

Some common seizure syndromes include:

i. Autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE) is an idiopathic localization-related epilepsy that is an inherited epileptic disorder that causes seizures during sleep. Onset is usually in childhood. These seizures arise from the frontal lobes and consist of complex motor movements, such as hand clenching, arm raising/lowering, and knee bending. Vocalizations such as shouting, moaning, or crying are also common. ADNFLE is often misdiagnosed as nightmares. ADNFLE has a genetic basis. (Bertrand *et al.*, 2002).

ii. Benign centrotemporal lobe epilepsy of childhood or benign Rolandic epilepsy is an idiopathic localization-related epilepsy that occurs in children between the ages of 3 and 13 years, with peak onset in prepubertal late childhood. Apart from their seizure disorder, these patients are otherwise normal. This syndrome features simple partial seizures that involve facial muscles and frequently cause drooling. Although most episodes are brief, seizures sometimes spread and generalize. Seizures are typically nocturnal and confined to sleep. The EEG may demonstrate spike discharges that

occur over the centrotemporal scalp over the central sulcus of the brain (the Rolandic sulcus) that are predisposed to occur during drowsiness or light sleep. Seizures cease near puberty (Loiseau *et al.*, 1988).

iii. Benign occipital epilepsy of childhood (BOEC) is an idiopathic localization-related epilepsy and consists of an evolving group of syndromes. It includes two subtypes, an early subtype with onset between three and five years, and a late onset between seven and 10 years. Seizures in BOEC usually feature visual symptoms such as scotoma or fortifications (brightly colored spots or lines) or amaurosis (blindness or impairment of vision). Convulsions involving one half the body, hemiconvulsions, or forced eye deviation or head turning are common. Younger patients typically experience symptoms similar to migraine with nausea and headache, and older patients typically complain of more visual symptoms. The EEG in BOEC shows spikes recorded from the occipital (back of head) regions. The EEG and genetic pattern suggest an autosomal dominant transmission (Kuzniecky, 1987). Lately, a group of epilepsies termed Panayiotopoulos syndrome (Panayiotopoulos, 2000) that share some clinical features of BOEC but have a wider variety of EEG findings are classified by some as BOEC.

iv. Catamenial epilepsy (CE) is when seizures cluster around certain phases of a woman's menstrual cycle.

v. Childhood absence epilepsy (CAE) is an idiopathic generalized epilepsy that affects children between the ages of 4 and 12 years of age, although peak onset is around five to six years old. These patients have recurrent absence seizures, brief episodes of unresponsive staring, sometimes with minor motor features such as eye blinking or subtle chewing. The EEG finding in CAE is generalized 3 Hz spike and wave

discharges. Some go on to develop generalized tonic-clonic seizures. This condition carries a good prognosis because children do not usually show cognitive decline or neurological deficits, and the seizures in the majority cease spontaneously with onging maturation.

vi. Ohtahara syndrome is a rare, but severe epilepsy syndrome usually starting in the first few days or weeks of life. The seizures are often in the form of stiffening spasms but other seizures including unilateral ones may be seen. The electroencephalogram (EEG) is characteristic. The prognosis is poor with about half of the infants dying in the first year of life; most if not all surviving infants are severely intellectually disabled and many have cerebral palsy. There is no effective treatment. A number of children have underlying structural brain abnormalities (Aicardi and Ohtahara, 2005).

vii. Primary reading epilepsy is a reflex epilepsy classified as an idiopathic localization-related epilepsy. Reading in susceptible individuals triggers characteristic seizures (Koutroumanidis *et al.*, 1998).

viii. Rasmussen's encephalitis is a symptomatic localization-related epilepsy that is a progressive, inflammatory lesion affecting children with onset before the age of 10. Seizures start as separate simple partial or complex partial seizures and may progress to epilepsia partialis continua (simple partial status epilepticus). Neuroimaging shows inflammatory encephalitis on one side of the brain that may spread if not treated. Dementia and hemiparesis are other problems. The cause is hypothesized to involve an immulogical attack against glutamate receptors, a common neurotransmitter in the brain (Rogers, 1994).

2.2.4 Pathophysiology of Epilepsy

Several genes that code for protein subunits of voltage-gated and ligand-gated ion channels have been associated with forms of generalized epilepsy and infantile seizure syndromes.

One speculated mechanism for some forms of inherited epilepsy are mutations of the genes that code for sodium channel proteins; these defective sodium channels stay open for too long, thus making the neuron hyper-excitable. Glutamate, an excitatory neurotransmitter, may, therefore, be released from these neurons in large amounts, which — by binding with nearby glutamatergic neurons — triggers excessive calcium (Ca^{2+}) release in these post-synaptic cells. Such excessive calcium release can be neurotoxic to the affected cell. The hippocampus, which contains a large volume of just such glutamatergic neurons (and NMDA receptors, which are permeable to Ca^{2+} entry after binding of both glutamate and glycine), is especially vulnerable to epileptic seizure, subsequent spread of excitation, and possible neuronal death. Another possible mechanism involves mutations leading to ineffective GABA (the brain's most common inhibitory neurotransmitter) action. Epilepsy-related mutations in some non-ion channel genes have also been identified (Miriam and Jennifer, 2005).

Several ligand-gated ion channels have been linked to some types of frontal and generalized epilepsies. One interesting finding in animals is that repeated low-level electrical stimulation to some brain sites can lead to permanent increases in seizure susceptibility: in other words, a permanent decrease in seizure "threshold." This phenomenon, known as kindling (by analogy with the use of burning twigs to start a larger fire). Chemical stimulation can also induce seizures; repeated exposures to some pesticides have been shown to induce seizures in both humans and animals. One mechanism proposed for this is called excitotoxicity (Miriam and Jennifer, 2005).

Physical, emotional, and social functioning of youth are interfered specifically if seizures are uncontrolledhttp://en.wikipedia.org/wiki/Epilepsy - cite_note-18 (Shafer, 2011). Some other noted consequences on repeated seizures are neuronal loss, gliosis, parenchymal microhemorrhages, excess of starch bodies, leptomeningeal thickening, subpial gliosis, perivascular gliosis and periavascular atrophyhttp://en.wikipedia.org/wiki/Epilepsy - cite_note-19 (Lopez, 2011).

2.2.5 Management of Epilepsy

Epilepsy is usually treated with medication prescribed by a physician; primary caregivers, neurologists, and neurosurgeons all frequently care for people with epilepsy. However, it has been stressed that accurate differentiation between generalized and partial seizures is especially important in determining the appropriate treatment http://en.wikipedia.org/wiki/Epilepsy - cite_note-pmid16382763-37 (Trost *et al.*, 2005).

Epilepsy surgery is an option for people with focal seizures that remain resistant to treatment (Duncan *et al.*, 2006). The goal for these procedures is total control of epileptic seizures (Birbeck, 2002), although anticonvulsant medications may still be required (Berg *et al.*, 2007).

Some natural products used in the management of convulsion include the following;

i. Dilipkumar Pal *et al.*, (2011) reported the ethanol extract of *Coccos nucifera root bark* significantly and dose dependently inhibited the onset and incidence of convulsions in mice.

ii. The methanol extract of *Tacazzea apiculata* root-bark was reported by Ahmed *et al.* (2009) to exhibit protection against the maximal electroshock test (MEST) in chicks that were induced convulsion by the (MEST), but did not protect them against PTZ-induced seizures.

iii. The hydro-ethanol extract of *Commiphora Africana* increased the threshold of PTZ-induced convulsion in mice and offered some level of protection against PTZ-induced convulsion in mice (Ezekiel *et al.*, 2010).

iv. Nwaiwu and Alkali (1986) also reported the anticonvulsant activity of the volatile oil from the fresh fruits of *Tetrapleura tetrapter* in mice, and the results obtained showed that the oil given intraperitoneally offered some protection against leptazol-induced convulsion.

v. The ethanol extract of the leaves of *Ocimum sanctum* was screened for its effect on central nervous system. It prolonged the time of lost reflex in mice due to pentobarbital, decreased the recovery and severity of electroshock and pentylenetetrazole-induced convulsions (Husain *et al.,* 2007).

vi. The extracts from *Tetrapleura tetraptera* exhibited anticonvulsant activity which could be linked to their ability to depress the central nervous system (Akah and Nwambie, 1994).

vii. *Bacopa monniera* extract also showed anticonvulsant action (Husain *et al.,* 2007).

2.3 Motor coordination

Motor coordination is realized by the nervous system at different levels. Concepts about the coordination within a limb define the controlled parameter in joint space, whereas concepts about the coordination between limbs stress the temporal control of schemas. Motor deficits of neurological patients point to the role of the different motor areas in motor coordination (Weiss and Jeannerod, 1998).

Examples of motor coordination are the ease with which people can stand up, pour water into a glass, walk, and reach for a pen. These are created reliably, proficiently and repeatedly, but these movements rarely are reproduced exactly in their motor details, such as movements in

joint angles when pointing (Domkin *et al.*, 2002) or standing up from sitting (Scholz and Schoner, 1999).

Picking up a glass of beer in a pub does not seem to be a difficult task. Yet, the apparent ease with which this action is executed obscures the complex tasks the motor system has to solve when organizing this drinking action. The motor system organizes the action at different levels. For the prehension movement to the glass, the reach has to be coordinated with the grasping component to bring the hand in the proper configuration to the right place (Jeannerod *et al.*, 1995). When one lifts the glass, the load and grip forces applied by the fingers have to be coordinated to account for the weight of the glass, its fragility, and possible slippage (Johansson and Cole, 1993). Furthermore, when one fills the glass from a bottle, the actions of the two arms must be coordinated. For example, the holding of the glass with one hand has to be functionally synchronized with the pouring action of the other hand (Weiss *et al.*, 1997). The coordination of the drinking action within the sequence of actions that precede or follow it takes place at another organizational level. The action structure is different whether simply drinking, clinking glasses, or passing the glass to somebody else (Weiss and Jeannerod, 1998).

Large numbers of musculoskeletal elements are involved in motor coordination. These different elements create many degrees of freedom (DoFs) by which any action can be done because of the range of ways of arranging, turning, extending and combining the various muscles, joints, and limbs in a motor task. Several hypotheses have been developed in explanation of how the nervous system determines a particular solution from a large set of possible solutions that can accomplish the task or motor goals equally well (Bernstein, 1967)

The coordination between limbs was mainly described within the temporal domain. The idea of a higher-order coordinating mechanism that temporally organizes lower-level elements

might explain not only the coordination between the reach and grasp components in prehension movements but also the synchronized activation of different body parts in pointing or the goal-directed temporal synchronization of the two hands in complex bimanual tasks (Weiss and Jeannerod, 1998).

2.3.1 Interlimb coordination

Inter-limb coordination concerns how movements are coordinated across limbs as proposed and modeled as coupled oscillators, a process that can be understood in the HKB (Haken, Kelso, and Bunz) model (Haken *et al.*, 1985).

Successful goal achievement in complex action involving different limbs requires precise temporal coordination. One of the basic principles that seems to operate at this organizational level is that of simultaneous onset (Weiss and Jeannerod, 1998). The coordination of complex inter-limb tasks is highly reliant on the temporal coordination. An example of such temporal coordination can be observed in the free pointing movement of the eyes, hands, and arms to direct at the same motor target. These coordination signals are sent simultaneously to their effectors. In bimanual tasks (tasks involving two hands), it was found that the functional segments of the two hands are tightly synchronized. One of the postulated theories for this functionality is the existence of a higher, "coordinating schema" that calculates the time it needs to perform each individual task and coordinates it using a feedback mechanism (Weiss *et al.*, 1997). There are several areas of the brain that are found to contribute to temporal coordination of the limbs needed for bimanual tasks, and these areas include the premotor cortex(PMC), the parietal cortex, the mesial motor cortices, more specifically the supplementary motor area (SMA), the cingulate motor cortex (CMC), the primary motor cortex (M1), and the cerebellum (Swinnen *et al.*, 2009).

Motor function can be differentially affected depending on experimental parameters. For example, unilateral brain injury models often produce hemiparesis-like effects, which may be reflected by deficits in grip strength, balance, and turning behavior, or may induce forepaw flexion. Many drugs can have either sedative or stimulant properties. (Curzon *et al.*, 2008)

2.3.2 Structures involved in motor coordination

The **cerebellum** is a region of the brain that plays an important role in motor control. It is also involved in some cognitive functions such as attention and language, and in regulating fear and pleasure responses (Wolf *et al.*, 2009), but its movement-related functions are the most solidly established. The cerebellum does not initiate movement, but it contributes to coordination, precision, and accurate timing. It receives input from sensory systems of the spinal cord and from other parts of the brain, and integrates these inputs to fine tune motor activity (Fine *et al.*, 2002). Because of this fine-tuning function, damage to the cerebellum does not cause paralysis, but instead produces disorders in fine movement, equilibrium, posture, and motor learning (Fine *et al.*, 2002).

The cerebellum is located at the bottom of the brain, with the large mass of the cerebral cortex above it and the portion of the brainstem called the pons in front of it (Ghez and Fahn, 1985). Based on surface appearance, three lobes can be distinguished in the cerebellum, called the flocculonodular lobe, anterior lobe (above the primary fissure), and posterior lobe (below the primary fissure). These lobes divide the cerebellum from rostral to caudal (in humans, top to bottom). In terms of function, however, there is a more important distinction along the medial-to-lateral dimension. Leaving out the flocculonodular part, which has distinct connections and functions, the cerebellum can be parsed functionally into a medial sector called the spinocerebellum and a larger lateral sector called the cerebrocerebellum. A narrow strip of protruding tissue along the midline is called the vermis (Latin for "worm") (Ghez and Fahn, 1985).

The smallest region, the flocculonodular lobe, is often called the vestibulocerebellum. It is the oldest part in evolutionary terms (archicerebellum) and participates mainly in balance and spatial orientation; its primary connections are with the vestibular nuclei, although it also receives visual and other sensory input. Damage to it causes disturbances of balance and gait (Ghez and Fahn, 1985).

The medial zone of the anterior and posterior lobes constitutes the spinocerebellum, also known as paleocerebellum. This region of the cerebellum functions mainly to fine-tune body and limb movements. It receives proprioception input from the dorsal columns of the spinal cord (including the spinocerebellar tract) and from the trigeminal nerve, as well as from visual and auditory systems. It sends fibres to deep cerebellar nuclei that, in turn, project to both the cerebral cortex and the brain stem, thus providing modulation of descending motor systems (Ghez and Fahn, 1985).

The lateral zone, constitutes the cerebrocerebellum, also known as neocerebellum receives input exclusively from the cerebral cortex (especially the parietal lobe) via the pontine nuclei (forming cortico-ponto-cerebellar pathways), and sends output mainly to the ventrolateral thalamus (in turn connected to motor areas of the premotor cortex and primary motor area of the cerebral cortex) and to the red nucleus (Ghez and Fahn, 1985). It is thought to be involved in planning movement that is about to occur (Kingsley, 2000), in evaluating sensory information for action (Ghez and Fahn, 1985), and in a number of purely cognitive functions as well (Timmann and Dauum, 2007).

The strongest clues to the function of the cerebellum have come from examining the consequences of damage to it. Animals and humans with cerebellar dysfunction show, above all, problems with motor control. They continue to be able to generate motor activity, but it loses precision, producing erratic, uncoordinated, or incorrectly timed movements. A

standard test of cerebellar function is to reach with the tip of the finger for a target at arm's length: A healthy person will move the fingertip in a rapid straight trajectory, whereas a person with cerebellar damage will reach slowly and erratically, with many mid-course corrections. Deficits in non-motor functions are more difficult to detect. Thus, the general conclusion reached decades ago is that the basic function of the cerebellum is not to initiate movements, or to decide which movements to execute, but rather to calibrate the detailed form of a movement (Ghez and Fahn, 1985).

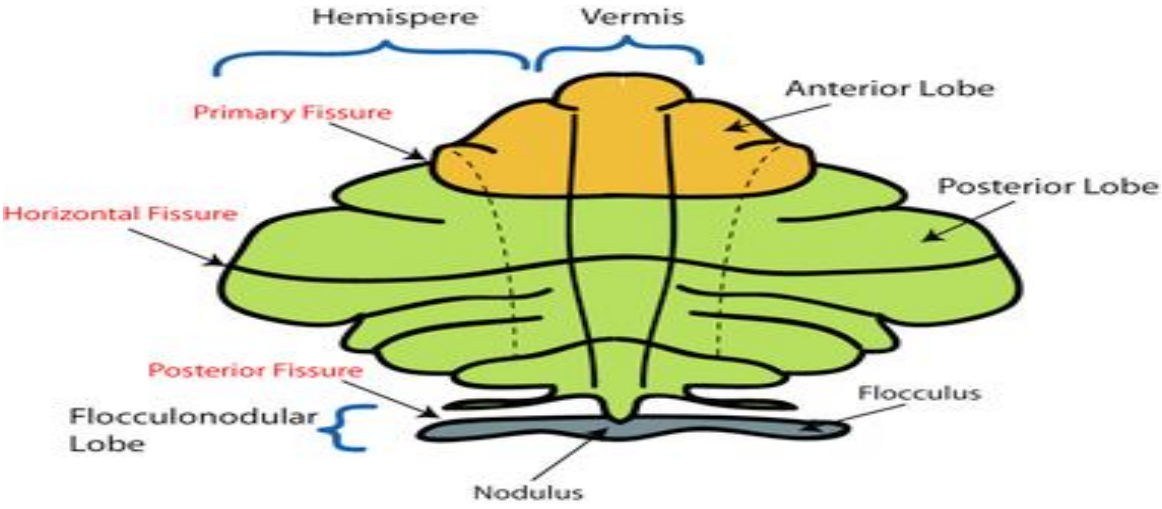

Figure 2.0: Schematic representation of the major anatomical subdivisions of the cerebellum. Superior view of an "unrolled" cerebellum, placing the vermis in one plane (Ghez and Fahn, 1985)

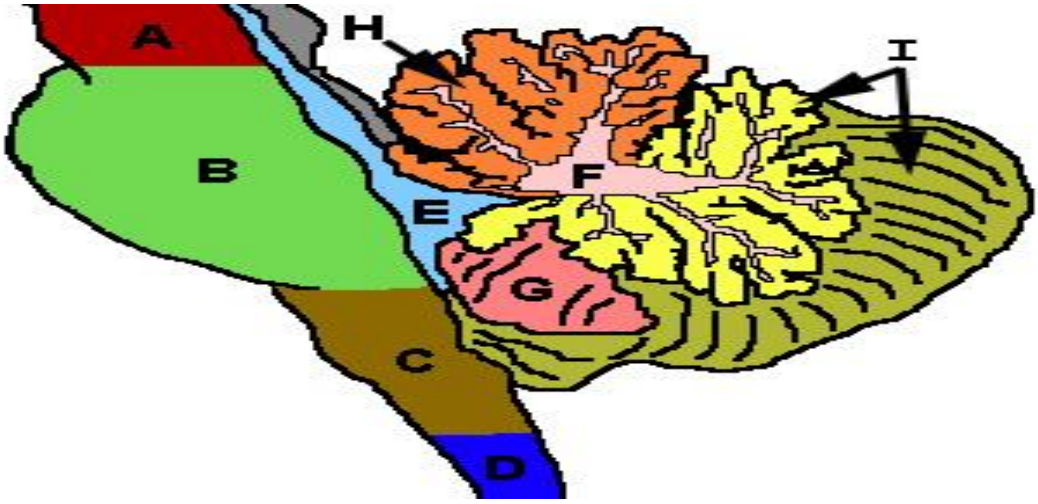

Figure 2.1: Cerebellum and surrounding regions; sagittal view of one hemisphere. A: Midbrain. B: Pons. C: Medulla. D: Spinal cord. E: Fourth ventricle. F: *Arbor vitae*. G: Tonsil. H: Anterior lobe. I: Posterior lobe (Ghez and Fahn, 1985).

The **cerebral cortex** is a sheet of neural tissue that covers the cerebrum and cerebellum. It is divided into left and right hemisphere. The cerebral cortex is where the information processing takes place. It plays a key role in memory, attention, perceptual awareness, thought, language, and consciousness. It is constituted of up to six horizontal layers, each of which has a different composition in terms of neurons and connectivity (Kandel *et al.,* 2000)

2.4 Anxiety and anxiety disorders

Anxiety is a psychological and physiological state characterized by somatic, emotional, cognitive, and behavioral components (Seligman *et al.*, 1995). It is the displeasing feeling of fear and concern (Davison, 2008). Anxiety can create feelings of fear, worry, uneasiness, and dread (Bouras and Holt, 2007). It may help an individual to deal with a demanding situation by prompting them to cope with it. When anxiety becomes excessive, it may fall under the classification of an anxiety disorder (National Institute of mental Health, 2008). Anxiety

symptoms are appropriate responses to stressful events or situations and often improve spontaneously, particularly if they are mild and of recent onset.

Anxiety disorders affect anywhere between 8% to 18% of the general population in any given year, with a lifetime prevalence exceeding 28% (Strine *et al.*,2008; Roy-Byrne *et al.*, 2009). Anxiety is the most common mental illness in America as approximately 40 million adults are affected by it (Robin, 2009).

Anxiety disorders take several forms: phobia, social anxiety, obsessive-compulsive, and post-traumatic stress. The physical effects of anxiety may include heart palpitations, tachycardia, muscle weakness and tension, fatigue, nausea, chest pain, shortness of breath, headache, stomach aches, or headaches. As the body prepares to deal with a threat, blood pressure, heart rate, perspiration, blood flow to the major muscle groups are increased, while immune and digestive functions are inhibited (the *fight or flight* response). External signs of anxiety may include pallor, sweating, trembling, and pupillary dilation. Someone who has anxiety might also experience it subjectively as a sense of dread or panic (Robin, 2009).

2.5 Pathophysiology of anxiety

The amygdala, an almond-shaped mass of nuclei located deep within the medial temporal lobes of the brain, plays a pivotal role in threat processing. It is generally regarded as fundamental for the acquisition of conditioned fear and for the expression of innate and learned fear responses. Efferent neurons emerging from the amygdala activate the sympathetic nervous system, thus driving the classic fightor- flight responses in end organs, such as increased heart rate and blood pressure, and pupillary and bronchodilation (Bandelow, 2002).

Functional neuro-imaging studies have demonstrated excessive arousal of the amygdala in patients suffering a range of anxiety disorders and this hyperactivity has been hypothesised as ontributing to the hypervigilant monitoring of negative information reported in these disorders (Whalen *et al.*, 2002; Dreverts, 2003). Other limbic structures, particularly the hippocampus, the medial prefrontal cortex and the locus coeruleus (which receives information directly from the sensory cortex and is largely under noradrenergic control) are also implicated in anxiety disorders (Bandelow, 2002).

At a molecular level, there is evidence of an underlying dysfunction of the neuro-inhibitory γ-aminobutyric acid (GABA) and serotonin (5-HT) neurotransmitter systems. Anxiety may therefore be reduced, either by increasing the effects of GABA with anticonvulsants or by increasing serotonin with antidepressants. Both are effective strategies for anxiolysis, albeit through different pathways (Nutt, 2005).

Of note is that projections from the amygdala to the locus coeruleus form an intersection of many diverse neurochemicals, which have increasingly become the focus of research seeking rational targets for the pharmacological treatment of anxiety disorders (Bandelow, 2004).

2.6 Benzodiazepines and plant extracts used in anxiety

A benzodiazepine (BZD is a psychoactive drug whose core chemical structure is the fusion of a benzene ring and a diazepine ring (Shorter, 2005).

Benzodiazepines enhance the effect of the neurotransmitter gamma-aminobutyric acid (GABA), which results in sedative, hypnotic (sleep-inducing), anxiolytic (anti-anxiety), anticonvulsant, muscle relaxant and amnesic action (Page *et al.*, 2002). These properties make benzodiazepines useful in treating anxiety, insomnia, agitation, seizures, muscle spasms, alcohol withdrawal and as a premedication for medical or dental procedures (Olkkola and Ahonen, 2008). Benzodiazepines are categorized as either short, intermediate or long-

acting. Short- and intermediate-acting benzodiazepines are preferred for the treatment of insomnia; longer-acting benzodiazepines are recommended for the treatment of anxiety (Dikeos *et al.*, 2008).

In general, benzodiazepines are safe and effective in the short term, although cognitive impairments and paradoxical effects such as aggression or behavioural disinhibition occasionally occur (Saias, 2008). Long-term use is controversial due to concerns about adverse psychological and physical effects, increased questioning of effectiveness and because benzodiazepines are prone to cause tolerance, physical dependence, and, upon cessation of use after long term use, a withdrawal syndrome (Lader, 2008; Lader *et al.*, 2009). Due to adverse effects associated with the long-term use of benzodiazepines, withdrawal from benzodiazepines, in general, leads to improved physical and mental health (Ashton, 2004; 2005). The elderly are at an increased risk of suffering from both short- and long-term adverse effects (Ashton, 2005; McIntosh *et al.*, 2005).

Benzodiazepines can be taken in overdoses and can cause dangerous deep unconsciousness. However, they are much less toxic than their predecessors, the barbiturates, and death rarely results when a benzodiazepine is the only drug taken. When combined with other central nervous system depressants such as alcohol and opiates, the potential for toxicity increases (Fraser, 1998). Benzodiazepines are commonly misused and taken in combination with other drugs of abuse (Charlson *et al.*, 2009).

Salahdeen and Yemitan (2006) reported that the aqueous leaf extract of *Bryophyllum pinnatum* caused a significant decrease in exploratory behaviour in mice, thus indicating that the plant may possess some CNS depressant activity (Lokesh *et al.,* 2009). Ethanolic root extract of *Coccos nucifera* has been reported to cause depressed awareness and alertness in mice (Dilipkumar *et al.*, 2011). The result of the study of the alcoholic extract of the

Dichrostachys cinerea root indicates a significant decrease in spontaneous motor activity and alteration of general behaviour (Ramya and Thaakur, 2009).

2.7 Mechanism of action of benzodiazepines

Benzodiazepines work by increasing the efficiency of a natural brain chemical, GABA, to decrease the excitability of neurons. This reduces the communication between neurons and, therefore, has a calming effect on many of the functions of the brain. The major molecular targets of marketed anticonvulsant drugs are voltage-gated sodium channels and components of the GABA system, including $GABA_A$ receptors, the GAT-1 GABA transporter, and GABA transaminase. Additional targets include voltage-gated calcium channels, SV2A, and α2δ (Meldrum and Rogawski, 2007; Rogawski and Bazil, 2008).

GABA controls the excitability of neurons by binding to the $GABA_A$ receptor (Olsen and Betz, 2006). The $GABA_A$ receptor is a protein complex located in the synapses of neurons. All $GABA_A$ receptors contain an ion channel that conducts chloride ions across neuronal cell membranes and two binding sites for the neurotransmitter gamma-aminobutyric acid (GABA), while a subset of $GABA_A$ receptor complexes also contain a single binding site for benzodiazepines. Binding of benzodiazepines to this receptor complex promotes binding of GABA, which in turn increases the conduction of chloride ions across the neuronal cell membrane. This increased conductance raises the membrane potential of the neuron, resulting in inhibition of neuronal firing. In addition, different $GABA_A$ receptor subtypes have varying distributions within different regions of the brain and, therefore, control distinct neuronal circuits. Hence, activation of different $GABA_A$ receptor subtypes by benzodiazepines may result in distinct pharmacological actions (Rudolf and Mohler, 2006). In terms of the mechanism of action of benzodiazepines, their similarities are too great to separate them into individual categories such as anxiolytic or hypnotic. For example, a hypnotic administered in

low doses will produce anxiety-relieving effects, where as a benzodiazepine marketed as an anti-anxiety drug will at higher doses induce sleep (Puri and Tyrer, 1998).

Once bound to the benzodiazepine receptor, the benzodiazepine ligand locks the benzodiazepine receptor into a conformation in which it has a greater affinity for the GABA neurotransmitter. This increases the frequency of the opening of the associated chloride ion channel and hyperpolarizes the membrane of the associated neuron. The inhibitory effect of the available GABA is potentiated, leading to sedatory and anxiolytic effects. Furthermore, different benzodiazepines can have different affinities for BzRs made up of different collection of subunits. For instance, those with high activity at the α_1 are associated with stronger hypnotic effects, whereas those with higher affinity for $GABA_A$ receptors containing α_2 and/or α_3 subunits have good anti-anxiety activity (Hevers and Luddens, 1998).

The benzodiazepine class of drugs also interact with peripheral benzodiazepine receptors. Peripheral benzodiazepine receptors are present in peripheral nervous system tissues, glial cells, and to a lesser extent the central nervous system (Arvat *et al.*, 2002). These peripheral receptors are not structurally related nor coupled to $GABA_A$ receptors. They modulate the immune system and are involved in the body response to injury (Zavala, 1997; Zisterer and Williams, 1997). Benzodiazepines also function as weak adenosine reuptake inhibitors. It has been suggested that some of their anticonvulsant, anxiolytic and muscle relaxant effects may be in part mediated by this action (Narimatsu *et al.*, 2006).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Plant Material Identification

Fresh leaves of *Bryophyllum pinnatum* were collected around Zaria, northern Nigeria. The identification and authentication of the plant was carried out at the Herbarium unit of the Department of Biological Science, Ahmadu Bello University, Zaria, where a specimen was deposited with a voucher number, 1834.

3.2 Preparation of the plant extract

The fresh leaves of *Bryophyllum pinnatum* were collected and dried under shade and ground into powder. The powder (407.23g) was macerated in 30 % of distilled water and 70 % methanol at room temperature for 24 hours. It was then filtered using a filtered paper (Whatmann size no.1), and the filtrate evaporated to dryness in water bath at 60°C. A greenish residue weighing 11.93g was obtained. This was kept in air tight bottle in a refrigerator until used.

3.3 Experimental animals

A total of one hundred and eight (108) adult mice of both sexes weighing between 25 to 30g were used. They were obtained from the Animal House laboratory of the Department of Pharmacology and Clinical Pharmacy, ABU, Zaria, and were kept in the laboratory for 7 days before use. They were placed on standard feed and allowed free access to food and water.

3.4 Experimental procedure

(a) **Diazepam-induced sleep in mice**

The method described by Beretz *et al*. (1978) and modified by Rakotonirina *et al.* (2001) was adopted in this study. 25 Swiss adult albino mice of either sex were divided into 4 groups of five mice in each group. The first group served as the control, second, third and fourth

groups were administered the extract of *Bryophyllum pinnatum* at the doses of 25, 50 and 100 mg/kg intraperitoneally respectively. Thirty minutes later, diazepam at a dose of (3 mg/kg) was administered to all the mice intraperitoneally. Each mouse was then observed for the onset and duration of sleep. The criterion for sleep is the loss of wrightening reflex, in which the mice cannot roll back when turned over (Miya *et al*., 1973).The interval between loss and recovery of wrightening reflex was used as the index of hypnotic effect (Fujimori, 1965; Soulimani *et al.,* 2001).

(b) **Pentylenetetrazole (PTZ) test**

The method of Swinyard *et al.* (1989) was employed. Clonic seizures were induced in male mice by the intrapreitoneal injection of 8.5 mg/kg Pentylenetetrazole (PTZ).

25 Swiss albino adult mice were divided into 5 groups of 5 mice each. Group 1 received normal saline intraperitonealy. Groups 2, 3 and 4 received the extract at the doses of 25, 50, and 100 mg/kg intraperitonealy respectively. Group 5 received valproic acid 20 mg/kg. The protective effect of the plant was recorded in mice treated 30 minutes before with the extract. The time of onset of seizures in non-protected mice was also recorded. The general clonus was characterized by forelimb clonus followed by full clonus of the body. The time taken before the onset of clonic convulsions, the duration of clonic convulsions, and the percentage of seizure and mortality protection were recorded (Vogel *et al*., 1997).

(c) **Test for exploratory activity in mice (Hole Board Test)**

Twenty five (25) mice were randomly divided into five groups of 5 mice each. The first group was administered normal saline which served as a negative control. Mice in the second group received diazepam 1.5mg/kg which served as a positive control, while third and fourth and fifth groups received the extract at the doses of 25mg/kg, 50mg/kg and 100mg/kg

respectively. The exploratory activities of the extract in mice following intraperitoneal administration was determined using the hole board test (File and Wardil, 1975).

The apparatus consists of a white wooden board (40 x 40cm) with four equidistant holes (1cm diameter x 2cm depth). Each mouse was placed singly at one corner of the board. It was allowed to move about and dip its head into the holes. Poking the nose into a hole is a typical behaviour of the mouse indicating a certain degree of curiosity. The number of dips in five minutes (enough time to exhibit curiosity otherwise) was recorded. The test was carried out 30 minutes after intraperitoneal treatment with the extract at the doses 25mg/kg, 50mg/kg and 100mg/kg

(d) **Mouse Beam Walk Assay**

This method offers improved sensitivity over the mouse Rota rod in determining motor coordination deficits induced by psychotropic agents (Stanley *et al.,* 2005). Mice were allowed to walk from a start platform along a ruler (80cm long and 83cm wide) elevated 30cm above the bench by metal supports to a goal box. Several trials were performed for each mouse and designed such that the mice tested are aware that there is a goal box that could be reached. A ruler was used because the mice find it easy to cross and at the same time, it will induce minimum anxiety (Stanley *et al.,* 2005).

Twenty five (25) mice were randomly divided into five groups of 5 mice each. The first group received normal saline which served as a negative control. Mice in the second group received diazepam 1.5mg/kg which served as a positive control, while third, fourth and fifth groups received the extract at doses of 25mg/kg, 50mg/kg and 100mg/kg. Once the animals were tested on the ruler, they were moved immediately to the beam test. The beam is made of wood, 8mm in diameter, 60cm long and elevated 30cm above the bench by a metal support. The animals were placed at one end of the beam and allowed to walk to the goal box thirty

minutes after treatment with the extract. Mice that fell were returned to the position they fell from with a maximum time of 60 seconds allowed on the beam. The number of foot slips (one or both hind limbs slipping from the beam was recorded with the aid of tally counter. The number of foot slips is a measure of motor coordination deficit (Stanley *et al.,* 2005).

Figure 3.1: The beam walk apparatus for determining motor coordination deficit in experimental animals. A goal box (hamster house) is to be attached at one end. The apparatus was constructed based on description by Stanley et al., (2005).

3.5 Chemicals used

All chemicals and drugs used were of analytical grade. Diazepam was purchased from La Roche Ltd. Basel, Switzerland.

3.6 Acute toxicity studies of the extract of *Bryophyllum pinnnatum* (leaves) on mice.

The intraperitoneal median lethal dose (LD_{50}) of the plant extract was conducted and calculated in mice according to the method of Lorke (1983) using thirteen (13) mice. In the initial phase, three (3) groups of three mice each was treated with the extract at doses of 10mg/kg, 100mg/kg and 1000mg/kg body weight intraperitoneally and observed for signs of toxicity and death for 24 hours. In the second phase, animals were divided into four (4) groups each containing a mouse and were injected intraperitoneally with four more specific doses of the extract at 20mg/kg, 40 mg/kg, 80 mg/kg and 160 mg/kg based on the result of the first phase. The LD_{50} was calculated as the square root of the product of the maximum dose for all survival and minimum dose for all death.

3.7 Preliminary phytochemical screening.

The extract of the leaves *Bryophyllum pinnatum* was subjected to preliminary phytochemical screening tests according to the method described by Trease and Evans (1989).

3.8 Statistical analysis

The data obtained from the experiment were expressed as Mean ± SEM. The data was statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's Post-Hoc test.

The values of $p < 0.05$ were considered as significant (Duncan *et al.,* 1977).

CHAPTER FOUR

4.0 RESULTS

4.1 PRELIMINARY PHYTOCHEMICAL SCREENING

The preliminary photochemical screening of the hydro-methanol extract of *Bryophyllum pinnatum* conducted indicated the presence of tannins, saponins, flavonoids, cardiac glycosides, glycosides and sugars (carbohydrate).

4.2 ACUTE TOXICITY STUDIES (LD_{50})

Toxicity signs noticed upon administration of the extract include decrease motor activity, decreased food intake and death. The median lethal dose of extract in mice was calculated to be 400mg/kg body weight intraperitoneally. See tables 4.1 and 4.2

Table 4.1: The percentage mortality of the different doses of the extract of *Bryophyllum pinnatum* (leaves) administered intraperitoneally in mice during the first phase of acute toxicity study.

Group	Dose(mg/kg)	Deaths	Mortality (%)
1	10	0/3	0
2	100	2/3	66.67
3	1000	3/3	100

Table 4.2: The percentage mortality of the different doses of the extract of *Bryophyllum pinnatum* (leaves) administered intraperitoneally in mice during the second phase of acute toxicity study.

Group	Dose(mg/kg)	Deaths	Mortality (%)
1	20	0/1	0
2	40	0/1	0
3	80	0/1	0
4	160	0/1	0

4.3 Neurobehavioural assessment

4.3.1 Beam walk assay

The administration of 25mg/kg and 50mg/kg showed a statistical significant increase ($P<0.05$) in the foot slips when compared with control group that received normal saline. The diazepam group also increased the number of foot slips significantly ($P<0.05$). The extract also showed a statistical significant increase ($P<0.05$) in the time it took the mice to traverse the beam (latency) at doses 25mg/kg and 50mg/kg. The diazepam group also increased the latency period significantly ($P<0.05$). The extract at 100mg/kg increased both foot flips and latency but was not statistically significant when compared to the control. See figures 4.3a and 4.3b and appendix I.

4.3.2 Explorative behaviour (Hole board test)

The extract at the different doses decreased the number of head dips in mice when compared to the control. Diazepam also caused a decrease in the number of head dips when compared to the control. Results obtained from the different doses of the extract and Diazepam however did not show any statistical significant decrease in the parameter been investigated as indicated by figure 4.4 and appendix II.

4.3.3 Diazepam-induced sleeping time

The extract at the different doses decreased the onset of sleep in mice but statistically and significantly ($p<0.05$) prolonged the duration of sleep in mice as evident in 25, 50 and 100mg/kg with corresponding duration of 218.20 ± 60.24, 269.60 ± 53.07 and 298.20±54.37 minutes respectively. The duration of sleep was dose dependent, with the 100mg/kg producing the highest duration of 298.20 ±54.37 minutes. See figures 4.5a and 4.5b and also appendix III.

4.3.4 Pentylenetetrazole-induced seizures test

The extract at doses 25 and 100mg/kg prolonged the onset of convulsion in the mice when compared with the control. These doses also protected the mice from death when compared with the control. The extract showed a biphasic activity by prolonging the onset of convulsion and increasing protection at the doses of 25 and 100mg/kg but less effective at 50mg/kg as shown in figures 4.6a and 4.6b as well as in appendix IV.

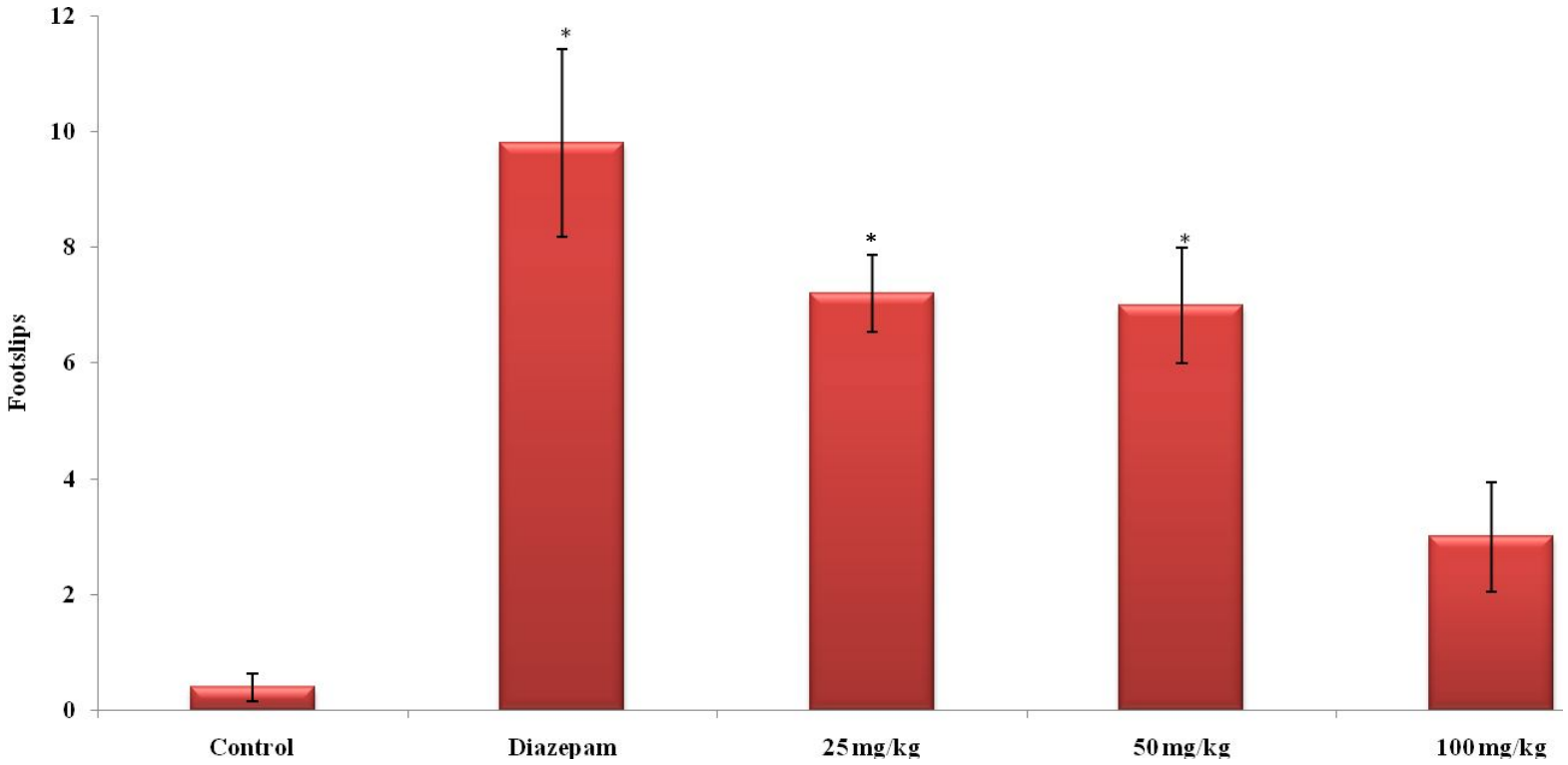

Figure 4.3a: *Bryophyllum pinnatum* effect on number of foot slips in mice. Values are expressed as mean ± SEM (n=5). $p<0.05$ compared with the control.

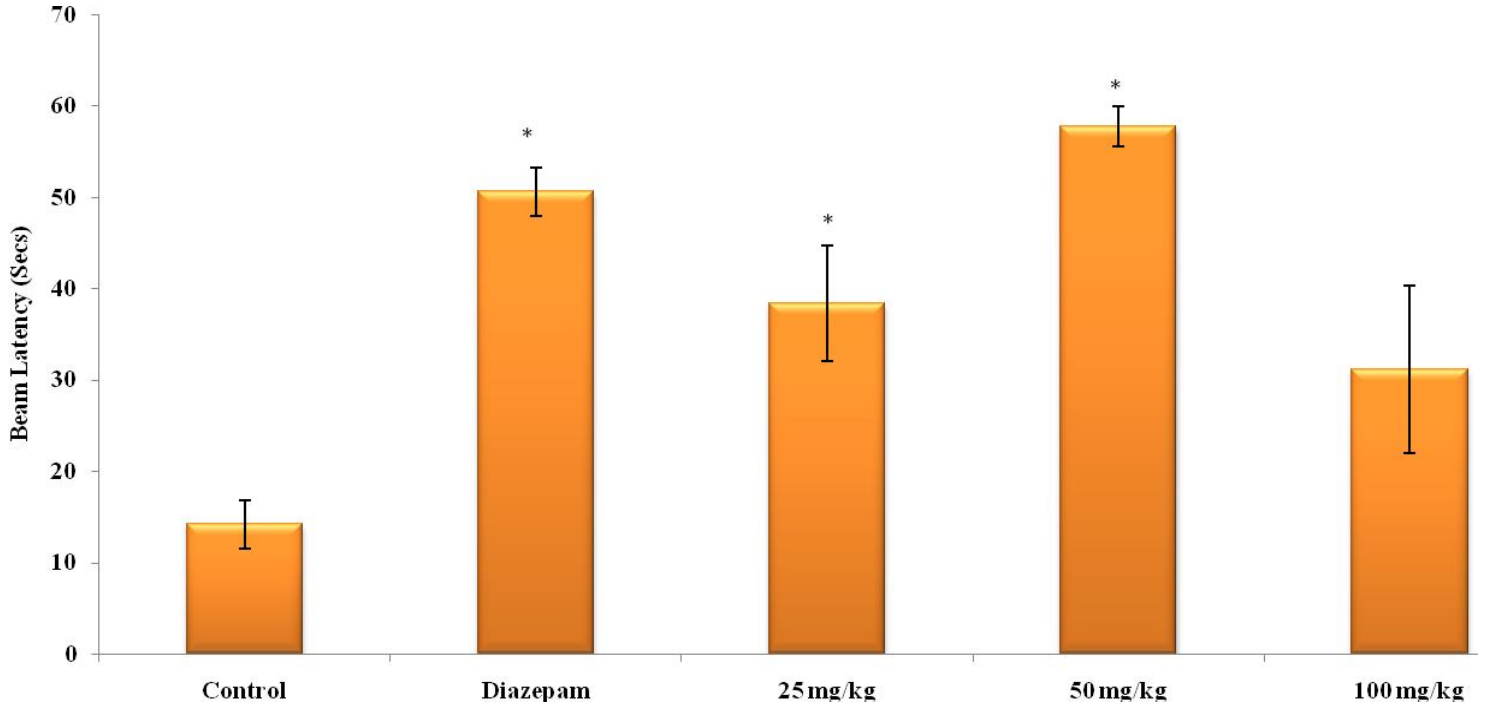

Figure 4.3b: *Bryophyllum pinnatum* effects on Beam latency in mice. Values are expressed as mean ± SEM (n=5). P<0.05 compared with the control.

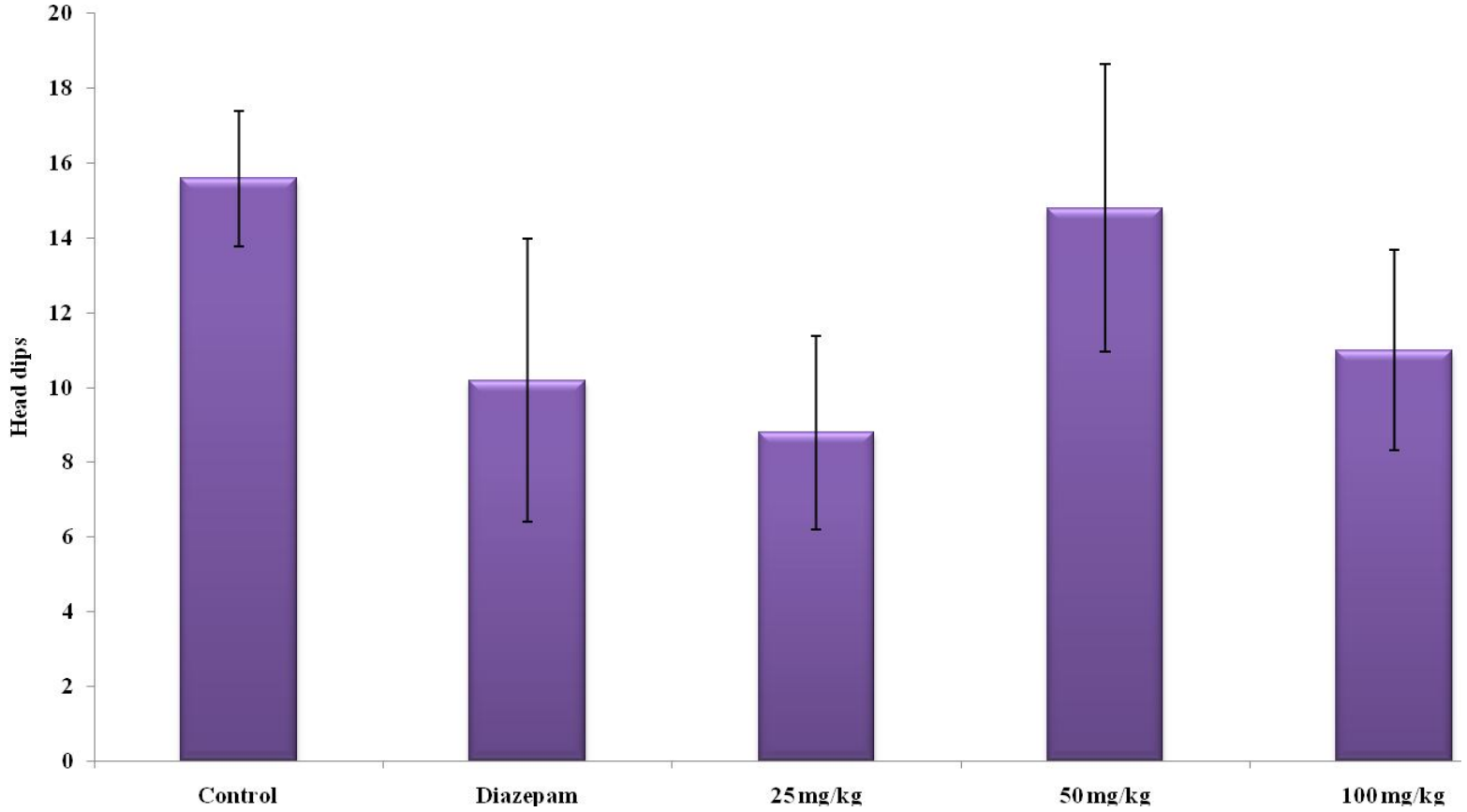

Figure 4.4: Mean values of head dips in five minutes due to the effect of *Bryophyllum pinnatum in mice.* Values are expressed as mean ± SEM (n=5). $p<0.05$ compared with the control.

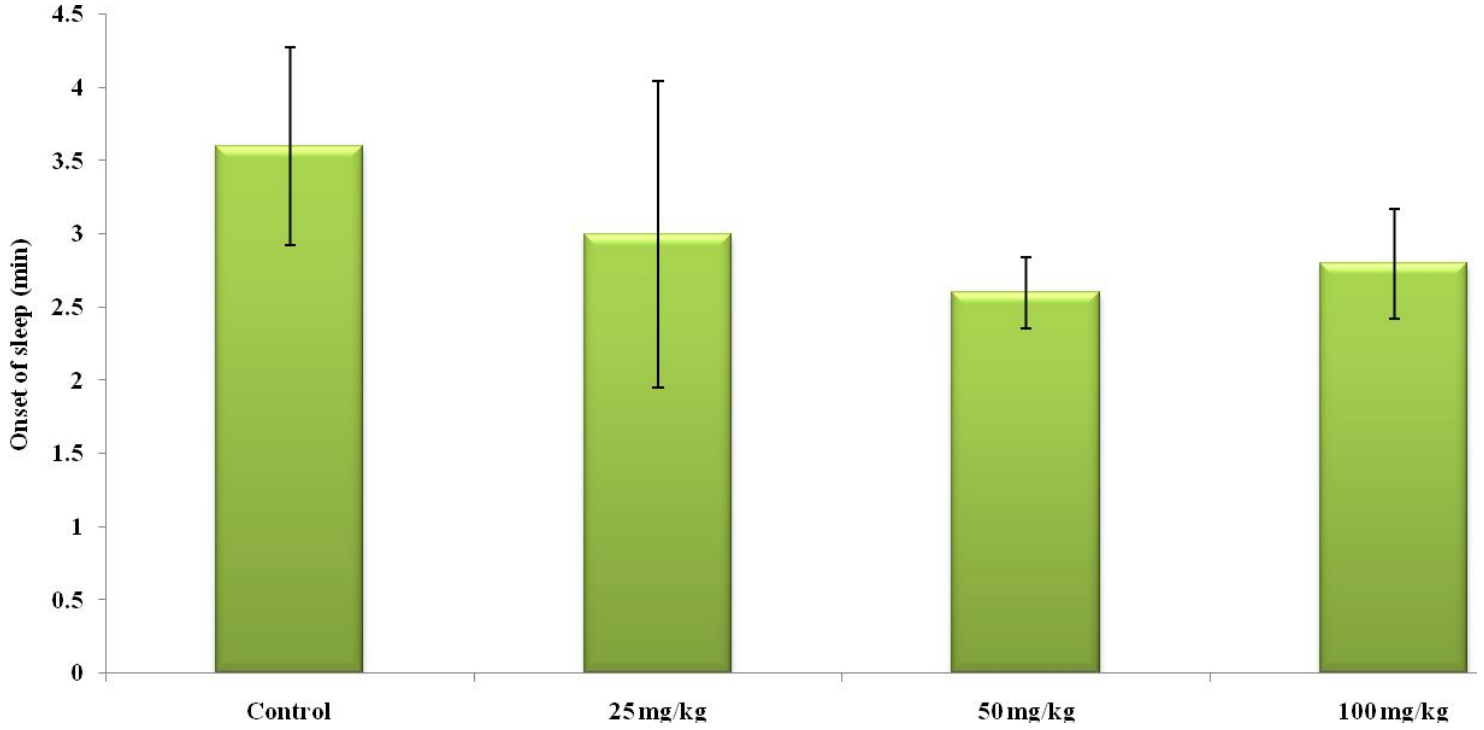

Figure 4.5a: Mean values of onset of sleep of *Bryophyllum pinnatum* on diazepam-induced sleep in mice. Values are expressed as mean ± SEM (n=5). $p<0.05$ compared with the control.

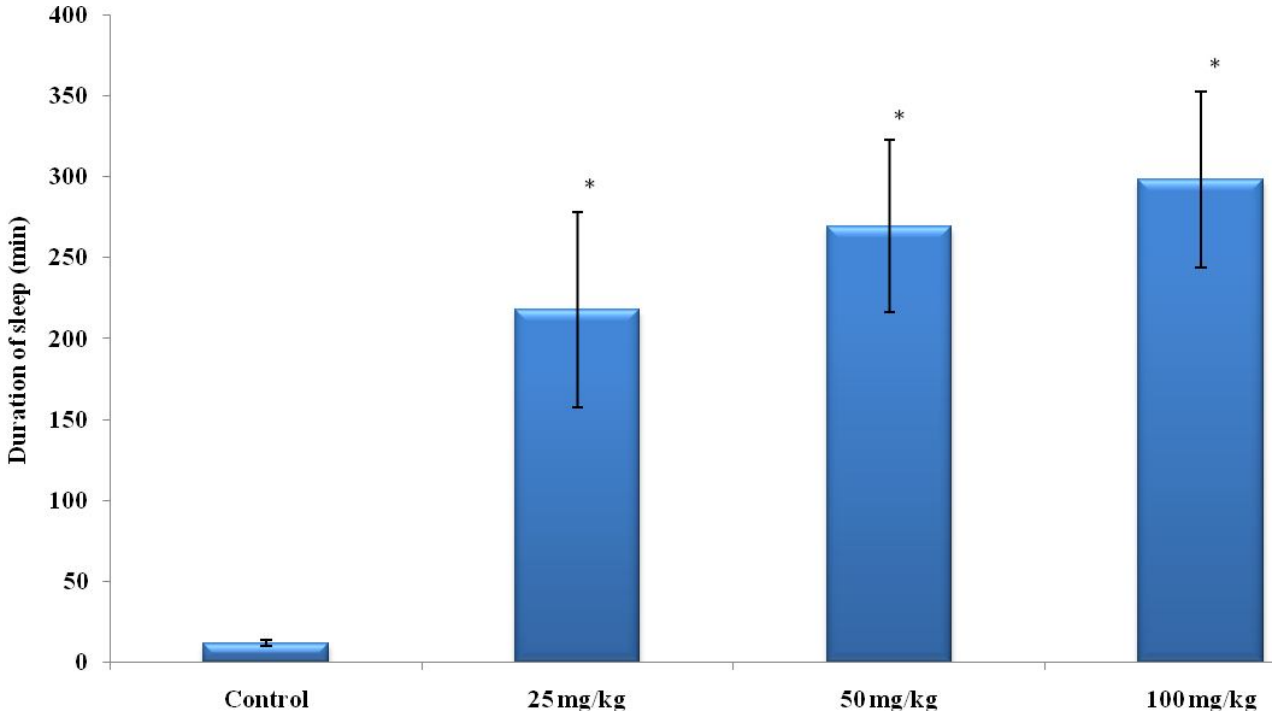

Figure 4.5b: Mean values of duration of sleep (min) of *Bryophyllum pinnatum* on diazepam-induced sleep in mice. Values are expressed as Mean ± SEM (n=5). *$p<0.05$ compared with the control.

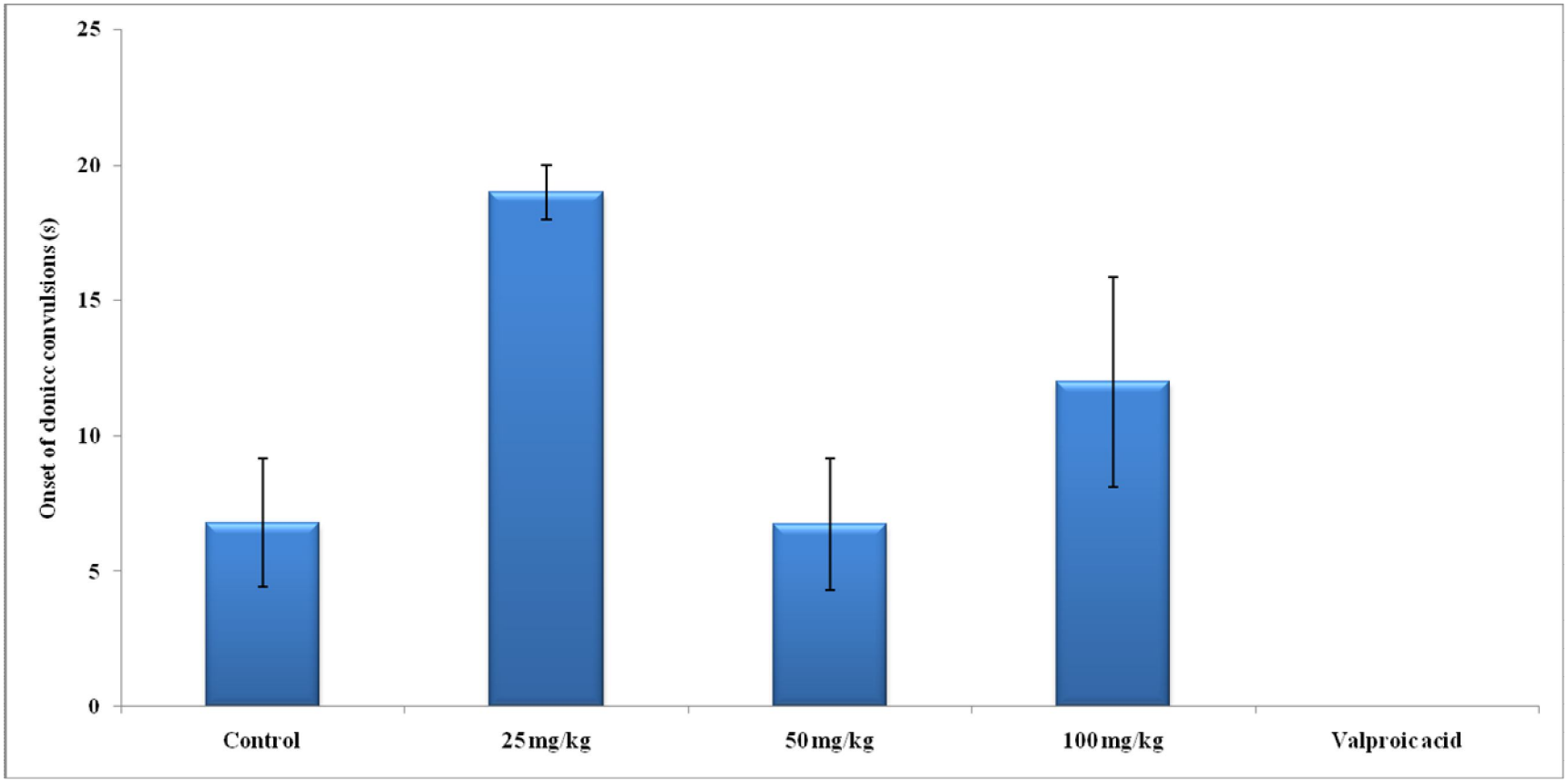

Figure 4.6a: Effect of hydro-methanol extract of *Bryophyllum pinnatum* on the onset of PTZ-induced convulsion in mice. Values are expressed as mean ± SEM (n=5). $p<0.05$ compared with the control.

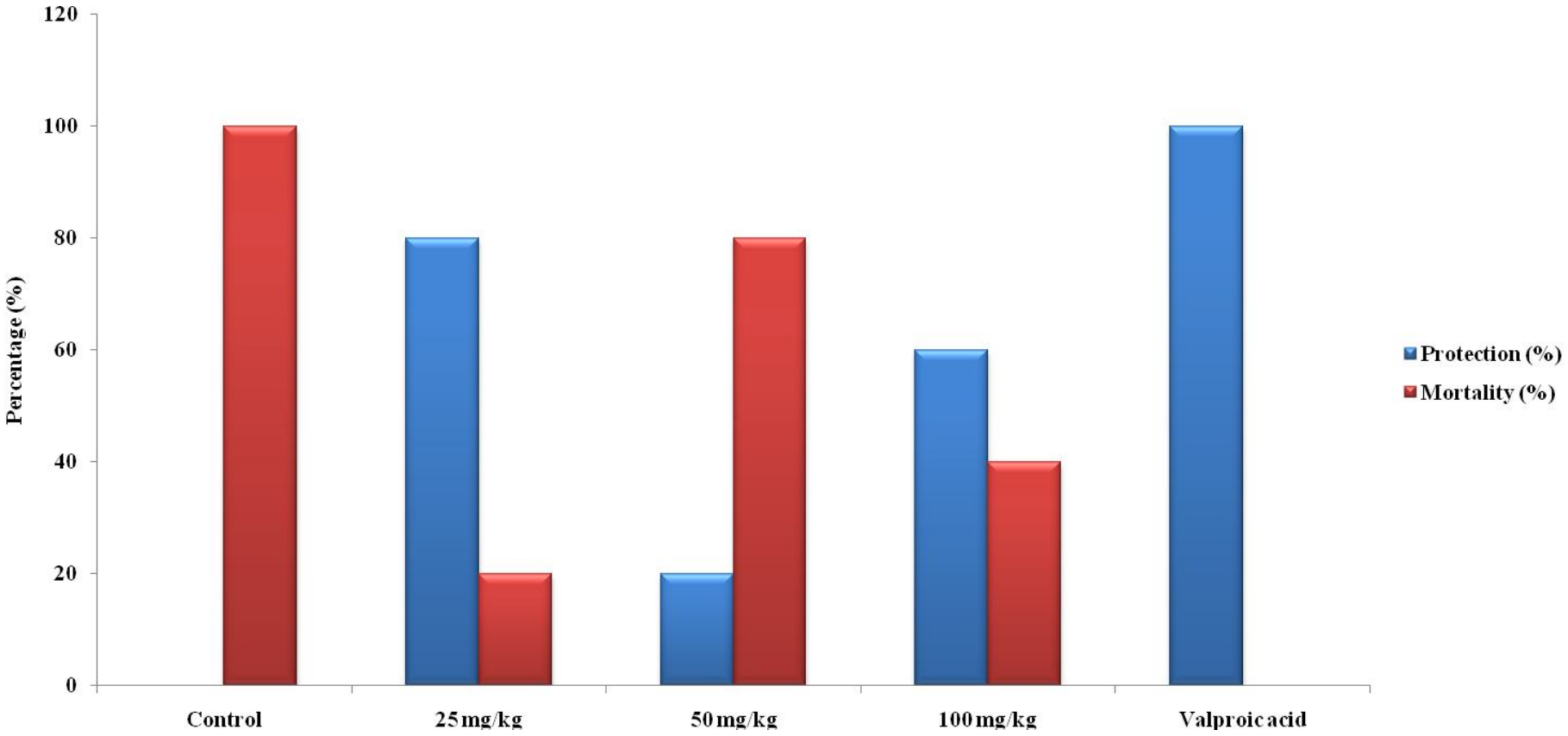

Figure 4.6b: Mean values of percentage protection and mortality due to the effect of hydro-methanol extract of *Bryophyllum pinnatum* on PTZ-induced convulsion in mice. Values are expressed as mean ± SEM (n=5).

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 DISCUSSION

The hydro-methanol leaf extract of *Bryophyllum pinnatum* significantly increased the number of foot slips and also the time taken to traverse the beam (latency) at lower and intermediate doses of 25 and 50mg/kg respectively. The frequency of foot slips observed have been found to be a sensitive measure of determining benzodiazepines-induced motor coordination deficits (Stanley *et al.,* 2005). The increase in the number of foot slips observed in this study suggests that the extract may possess a potent sedative property.

The results obtained showed that the extract at the different doses decreased the number of head dips in mice when compared with the control, though it was not statistically significant. File and Wardill (1975) reported that the hole-board experiment is a measure of exploratory behavior in animals. A decrease in this parameter reveals a sedative behaviour and a high propensity for antipsychotic action (Feilding and Lal, 1978; File and Pellow, 1985). This parameter has been accepted for the evaluation of anxiety conditions in animals (Crawley, 1985). Decrease in exploratory behaviour in mice is a measure of CNS depression as demonstrated by the reduction in number of head dips (Adzu, 2002; Viswanatha *et al.,* 2006). This decrease in exploratory behavior further supports the neurosedative property of the extract as reported by Salahdeen and Yemitan, (2006) and its possible application in anxiety conditions.

The extract was observed to cause a decrease in the onset of sleep and significantly prolonged the duration of sleep in mice when statistically compared with the control. This effect was found to be dose dependent. Substances that potentiate diazepam-induced sleep in mice are said to possess sleep inducing properties (Guillemain and Tatau, 1980; Rakotonirina *et al.,* 2001) It may

be suggested that the extract possesses sleep inducing properties since it potentiated diazepam-induced sleep in the mice. Prolongation of diazepam induced sleep strongly suggests central depressant activity of the extract (Perez *et al*., 1998). Sedative-hypnotic agents act to increase GABA mediated synaptic inhibition either by directly activating the GABA receptors or more usually by enhancing the action of $GABA_A$ receptors. Benzodiazepines and barbiturates are examples of widely used therapeutic agents that act as positive allosteric modulators at GABA receptors (Johnston, 2005). The ability of the extract to potentiate the sedative property of diazepam suggests that it may possibly act by interacting with GABA-mediated synaptic transmission.

Diazepam act selectively on $GABA_A$ receptor which mediates fast inhibitory synaptic transmission throughout the CNS (Argyropoulos *et al*., 2000). Hypnotics and barbiturates bind to sites of GABA receptors complex, increasing Cl^- flow through ionophore, causing hyperpolarization and reduced cell firing. They may shut off neurons in the reticular activating system and inhibit transmission and activity of neurons that project to the cortex and thalamus (Rosenthal, 1998). It is also thought that benzodiazepines may in part exert their actions by blocking the reuptake of adenosine (Rosenthal, 1998).

The extract delayed the onset of convulsion in mice treated with 25mg/kg and 100mg/kg of the extract when compared with the control. It also increased the protection in the mice by 60% and 80% in 100mg/kg and 25mg/kg respectively, while in 50mg/kg, the protection was 20%. The delayed onset of convulsion and increased protection was not dose dependent. The ability of the extract to inhibit clonic seizures suggests anticonvulsant activity of *Bryophyllum pinnatum* for the treatment of seizures. The anticonvulsant activity of PTZ test identifies compounds that can

raise seizure threshold in the brain (White *et al.,* 1998). PTZ has been shown to interact with GABA receptor complex (DeDeyn *et al.,* 1992).

Ability of *Bryophyllum pinnatum* to inhibit clonic seizure in the PTZ test suggests that it may have the ability to raise seizure threshold. The effectiveness of a drug against PTZ-induced seizures indicate its probable effectiveness against seizures (McNamara, 1989). PTZ has been reported to inhibit chloride conductance by binding to picrotoxin sites of $GABA_A$ receptor complex (Krall *et al.,* 1978). PTZ may be exerting its convulsive effect by inhibiting the activity of GABA at $GABA_A$ receptors (De Sarro et al., 1999) the major inhibitory neurotransmitter which is implicated in epilepsy (Gale, 1992). The enhancement and inhibition of the neurotransmission of GABA will enhance and attenuate convulsion respectively (Meldrum, 1981; Gale, 1992; Westmorland *et al*., 1994). Phenobarbitone and Diazepam have been shown to exert their antiepileptic effects by enhancing the GABA-mediated inhibition in the brain (Porter and Meldrum, 2001). Antagonism of PTZ-induced seizures suggests effects on GABA-ergic neurotransmission (Ahmed *et al*., 2009).

Based on these results, it is therefore probable that the hydro-methanol leaf extract of *Bryophyllum pinnatum* has considerable antiepileptic action that might involve both GABA-nergic and glycinergic inhibitory mechanism. Saponins and flavonoids have been reported by several researchers (Woo *et al*., 1980; Dubios *et al*., 1986; Amos *et al*., 2001; Viswanatha *et al*., 2006; Musa *et al*., 2008) to be responsible for sedative and inhibition of spontaneous motor activity in mice.

Preliminary phytochemical investigations of *Bryophyllum pinnatum* revealed the presence of carbohydrates, flavonoids, tannins, saponins, glycosides and cardiac glycosides. Since *Bryophyllum pinnatum* exhibited anti-seizure activity, it might be clinically useful in the management of epilepsies.

5.2 CONCLUSION

The neuropharmacological investigation of the hydro-methanol leaf extract of *Bryophyllum pinnatum* showed that the extract possesses some CNS depressant activities as observed in the decreased head dipping in the Hole Board test and beam walk assay conducted. The extract also potentiated the total sleep time induced by diazepam and also showed anti-convulsant activity on pentylenetetrazole-induced seizures in mice. From above, the extract of *Bryophyllum pinnatum* may be inferred to contain some CNS depressant agents. The anti-anti-depressant activity of the extract may be suggested to be mediated through GABA-nergic transmission since substances that stimulate GABA release are known to possess CNS depressant abilities.

The sedative, anti-convulsive, decreased motor activity and decreased exploratory effects of *Bryophyllum pinnatum* might therefore be due to any one or combination of the phytochemicals present in the plant.

5.3 RECOMMENDATIONS

Based on the findings of this study, it is may be recommended that:

1. Further investigations be conducted to isolate the component(s) that may be responsible for these behaviours.

2. Chronic toxicity studies of this plant should be done so as to ascertain the safety of usage of this plant extract.

REFERENCES

Adewunmi, C.O., and Ojewole, J.A.O. (2004). Safety of tradition medicine, Complementary and alternative medicine in Africa. *African Journal of Tradition,* . 1: 1-3.

Adzu, S., Amos, S., Dzarma, C.W. and Gamaniel, K. (2002). Effect of Zizypus spinchristi wild aqueous extract on the central nervous system in mice. *Journal of Ethnopharmacology.* 79:13-16

Agoha, R.C. (1974). Medicinal Plants of Nigeria. Offset Drakkerij. Faculfcitder Wiskunde in Naturwetenschappen, the Netherlands p 41 and 33.

Ahmed A., Yaro A. H., Magaji M. G., Ibrahim H., Musa K. Y. and Abubakar L.Y. (2009). Anticonvulsant activity of Tacazzea apiculata oliv. (Periplocaceae). *Nigerian Journal of Pharmaceutical Sciences*. 8: 41- 46

Aicardi, J. and Ohtahara S. (2005). Severe neonatal epilepsies with suppression-burst pattern. Epileptic Syndromes in Infancy, Childhood and Adolescence (4th edition) Eds Roger J., Bureau M., Dravet C.,Genton P., Tassinari C. and Wolf P. John Libbey Eurotext

Akah, P.A. and Nwambie, A.I. (1994). Evaluaion of Nigerian traditional medicinal plants used for rheumatic (inflammatory) disorders. *Journal of Ethnopharmacology*, 42: 197-182.

Akinpelu, D.A. (2000). Anti-microbial activity of Bryophyllum pinnatum leaves. *Fitoterapia*; 71(2):193-194

Amos, S., E. Kolawale, P. Akah, C. Wambebe, and K. Gammaniel, (2001). Behavioural effect of the aqueous extract of *Guinea Senegalensis* in mice and rats. *Phytomedicine*, 8(5): 356 - 361.

Argyropoulos, S.V., J.J. Standford and D.J. Nutt, (2000). The Psychobiology of anxiolytic drugs. Part 2: pharmacological treatments of anxiolytic. *Pharmacology and Therapeutics.* 83: 213-227.

Arvat, E., Giordano R., Grottoli S. and Ghigo E. (2002). "Benzodiazepines and anterior pituitary function". *Journal of Endocrinological Investigation* 25 (8): 735–47.

Ashton, C.H. (2005). "The diagnosis and management of benzodiazepine dependence". *Current Opinions in Psychiatry* 18 (3): 249–55.

Ashton, H (2004). "Benzodiazepine dependence". In Haddad P, Dursun S, Deakin B (eds.). *Adverse Syndromes and Psychiatric Drugs: A Clinical Guide*. Oxford University Press. pp. 239–60.

Aston-Jones, G. and Bloom, F. E. (1981). Activity of norepinephrine- containing locus coeruleus neurons in behaving rats anticipates fluctuations in the sleep-waking cycle. *Journal of. Neurosciences*. 1: 876–886.

Attarian, H.P. (2003). Epidemiology of insomnia. In: *Current clinical neurology:Clinical Handbook of insomnia* (Attarian H.P. ed). Humana Press Inc., Totona, NJ. pp.11-21.

Aur, D. (2011). Understanding the Physical Mechanism of Transition to Epileptic Seizures, *Journal of Neuroscience Methods,* (200) Pp 80-85

Azubuike, J.C., and Nkanginieme, K.E.O. (1996). *Paediatrics and Child Health in a Tropical Region*. Fourth Dimension Publishers Enugu, 388 pp.

Bandelow, B., Zohar, J., Hollander, E., Kasper, S., and Möller, H.J. (2002). "World Federation of Societies of Biological Psychiatry (WFSBP) guidelines for the pharmacological treatment of anxiety, obsessive-compulsive and posttraumatic stress disorders". *World J. Biol. Psychiatry* 3 (4): 171–99

Balzer, G. (1949). Bryophyllum. Ein Beitrag zu seina Pflanzen-morphologie. Berlin-leinmachnow: Gartenverlag GmbH,

Benca, R. M., Obermeyer, W.H., Thisted, R.A. and Gillin, J.C. (1991). "Meta-analysis of sleep changes in psychiatric disorders," *Sleep Research.* 20, 169.

Beretz, A., Haag-Berrurier M., and Anton, R. (1979). Choix de Methods Pharmacologiques pour l'aubepine. Plantes Medicinales et Phytotherapic, 4th Ed., 143 pp.

Berg, A.T., Langfitt, J.T., Spencer, S.S. and Vickrey, B.G. (2007). "stopping antiepileptic drugs after epilepsy surgery: a survey of us epilepsy center neurologists". *Epilepsy Behav* 10 (2): 219–222

Bernstein, N. (1967). The Coordination and Regulation of Movements. Pergamon Press. New York.

Bertrand, D., Picard, F., Le Hellard, S., Weiland, S., Favre, I. and Phillips, H. (2002). "How mutations in the nAChRs can cause ADNFLE epilepsy". *Epilepsia* 43 Supple 5: 112–122

Biosecurity Queensland (2011). The University of Queensland. Special edition of Environmental Weeds of Australia for Biosecurity Queensland. http://www.dpi.qld.gov.au.

Bouras, N. and Holt, G. (2007). *Psychiatric and Behavioural Disorders in Intellectual and Developmental Disabilities* 2nd ed. Cambridge University Press: UK.

Bremer, F. (1935). Cerveau _isol__ et physiologie du sommeil. Comptes Rendus de Societe Biologie. (Paris) 118, 1235–1241.

Buffet-Jerrot, S.E. and Stewart, S.E. (2002). Cognitive and Sedative effects of Benzodiazepines Use. *Current Pharmaceutical Design* 8:4558.

Burkill, H. M. (1985). The Useful Plants of West Tropical Africa. 2nd Edn., Vol.1, Families A-D.

Calixto, J.B. (2000). Efficacy, safety, quality control, marketing and regulatory guidelines for herbal medicines (phytotherapeutic agents). *Brazil Journal of Medicinal Biology Research*. 33: 179-189.

Cardinale, V. (*1998.) Drug Topics Red Book*, Medical Economics Co., Inc., p. 102.

Charlson, F., Degenhardt, L., McLaren, J., Hall, W. and Lynskey, M. (2009). "A systematic review of research examining benzodiazepine-related mortality". *Pharmacoepidemiology of Drug Saf*ety. 18 (2): 93–103.

Chase, M. (1993). *Basics of Sleep Behavior,* Sleep Research Society ,UCLA and Sleep Research Society, Los Angeles CA

Chokroverty, S. (1999). *Physiological Changes in Sleep Disorders*. Boston: Butter worth Heinmann, pp. 95-126.

Chopra, R.N., Nayar, S.L. and Chopra, I.C. (1956). Glossary of Indian Medicinal Plants vol. 1. Council of Scientific and Industrial Research, New Delhi pp 330.

Chu., N. and Bloom, F. E. (1973). Norepinephrine-containing neurons: changes in spontaneous discharge patterns during sleeping and waking. *Science* 179, 908–910.

Costa, S., Jossang, A., and Bodo, B. (1995). Proprie´te´s biologiques et phytochimie des kalanchoe. In: Boiteau P, Allorge-Boiteau L, editors. Kalanchoe de Madagascar. *Syste´matique, e´cophysiologie et phytochimie.* Paris: Karthala;. p. 219–35.

Crawley, J.N. (1985). Exploratory behaviour models of anxiety in mice. *Neuroscience and Behavioral Review*: 9 :37-44

Cullinan, W. E. and Zaborszky, L. (1991). Organization of ascending hypothalamic projections to the rostral forebrain with special reference to the innervation of cholinergic projection neurons. *Journal of Comparative Neurology.* 306, 631–667.

Curzon, P., Min, Z., Richard, J. R., and Gerard B. F. (2008). The Behavioral Assessment of Sensorimotor Processes in the Mouse: Acoustic Startle, Sensory Gating, Locomotor Activity, Rotarod, and Beam Walking: *Methods of Behavior Analysis in Neuroscience*.CRC press pp 145-177

Daan, S., Barnes, B.M., and Strijkstra, A.M. (1991). "Warming up for sleep? Ground squirrels sleep during arousals from hibernation". *Neuroscience Letters.* 128 (2): 265–268.

Dahlstrçm, A. and Fuxe, K. (1964). Evidence for the existence of monamine-containing neurons in the central nervous system. I. Demonstration of monoamines in the cell bodies of brain stem neurons. *Acta Physiologica Scandinavica.* 62, 1–55.

Dalziel, J.M. (1955). The useful Plants of West Tropical Africa. Grown Agents for Oversea Governments and Administrations, London. pp. 28, 53, 415.

Datta, S. and Siwek, D. F. (2002). Single cell activity patterns of pedunculopontine tegmentum neurons across the sleep wake cycle in the freely moving rats. *Journal of Neuroscience Research*. 70, 611–621.

Davison, G. C. (2008). *Abnormal Psychology*. Toronto: *Veronica Visentin.* pp. 154.

De Deyn, P.P., D'Hoope, R., Marescau, B., and Pei,Y.Q (1992). Chemical model for epilepsy with some references to their applicability in the development of anticonvulsants. *Epilepsy Research,* 12: 87-110.

de Lecea, L., Kilduff, T. S., Peyron, C., Gao, X., Foye, P. E., and Danielson, P. E. (1998). The hypocretins: hypothalamusspecific peptides with neuroexcitatory activity. *Proceedings of the National Academy of Science.USA.* 95, 322–327.

De Sarro, A., Cecchetti, V., Fravolini, V., Naccari, F., Tabarrini, O., and De Sarro G.(1999). Effects of novel β-desfluroquinolones and classic quinolones on pentelenetetrazole-induced seizures in mice. *Antimicrobial Agents and Chemotherapeutics*.; 43: 1729-36.

Dikeos, D.G., Theleritis, C.G., and Soldatos, C.R. (2008). "Benzodiazepines: effects on sleep". In Pandi-Perumal SR, Verster JC, Monti JM, Lader M, Langer SZ (eds.). *Sleep Disorders: Diagnosis and Therapeutics*. Informa Healthcare. pp. 220–2

Dilipkumar, P., Abhijit, S., Sumanta, G., Sandip, J. and Soumit, M. (2011). CNS Depressant activities of roots of *Coccos nucifera* in mice, *Acta Poloniae Pharmaceutica - Drug Research,* 68: 249- 254

Dorr M., Joycee, D. Porsolt, R.D., Steinberg, H., Summerfield, A., and Tomkiewiez, M. (1971). Persistence of dose-related behaviour in mice. *Nature* 231:121-123

Dubois, M.A., Ilyas, M. and Wagnar, H. (1986). Cussonoides A and B, two Triterpenes-saponins from Cussonia barteri. *Planta Medica*, 56: 80-83

Duncan, J..S., Sander, J.W., Sisodiya, S.M., and Walker, M.C. (2006). "Adult epilepsy". *Lancet* 367 (9516): 1087–100

Duncan. R.C., Knapp. R.G., and Miller. M.C. (1977). Test of hypothesis in Population Means. *In Introductory Biostatistics for the Health Sciences*. John Wiley and Sons Incorporation, New York, 71-96 pp.

Engler, A. (1926). Die naturrlichen Pflanzenfamilien nebst ihren Gatttugen und wichtigsen Arten insbesondre der Nutpflanzen. Leipzig: W. Engelmann, 1926, p 402 – 12.

Ezekiel, I., Mabrouk, M.A., Ayo, J.O., Goji, A.D.T., Okpanachi, A.O., Mohammed, A. and Tanko, Y. (2010). Study of the Effect of Hydro-Ethanolic Extract of *Commiphora Africana* (Stem-bark) on Sleeping Time and Convulsion in Mice, *Asian Journal of Medical Sciences* 2(3): 85-88

Feilding, S.W. and Lal, H. (1978). Behavioural actions of neuroleptics. In:., Handbook of Psychopharmacology. Edited by Iversen L.L., Iversen, S.D. and Snyder, S.M, Vol. 10. Plenum Press, New York, pp: 91-128.

File, S. and Pellow, S. (1985).The effect of triazolobenzodiazepines in two animal tests of anxiety and on the hole–board. *British Journal of Pharmacology*. 86:729–735.

File, S.E and Wardill, A.G (1975). Validity of head dipping as a measure of explorating a modified hole-board. *Psychopharmacologia*, 44:53-59.

Fine, E.J., Ionita, C.C., and Lohr, L. (2002). "The history of the development of the cerebellar examination". *Seminars in Neurology* 22 (4): 375–84.

Fisher, R., van Emde, B. W., Blume, W., Elger, C., Genton, P., Lee, P., and Engel, J. (2005). "Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE)". *Epilepsia* 46 (4): 470–2.

Ford, B., Holmes, C. J., Mainville, L. and Jones, B. E. (1995). GABAergic neurons in the rat pontomesencephalic tegmentum: codistribution with cholinergic and other tegmental neurons projecting to the posterior lateral hypothalamus. *Journal of Comparative Neurology*. 363, 177–196.

Fraser, A.D. (1998). "Use and abuse of the benzodiazepines". *Therapeutic Drug Monitoring* 20 (5): 481–9.

Fujimori, H. (1995). Potentiation of barbital hyponosis as an evaluation method for CNS depressant. *Psychopharmacology*; 7: 374-377.

Gaillard, J.M., Nicholson, A.N. and Pascoe, P.A. (1989). "Neurotransmitter systems" in *Principles and Practice of Sleep Medicine,* (edit., Kryger, M.H., Roth, T., Dement, W.C.), W.B. Saunders and Co., Piladelphia PA pp. 202-212.

Gaind, K. and Gupta, R. (1972). Alkanols, triterpens and sterols of Kalanchoepinnata. *Phytochemistry* ;11:1500–2.

Gaind, K. and Gupta, R. (1974). Identification of waxes from the leaves of Kalan-choe pinnata. *Planta Medica*;23:193–7.

Gale, K.(1992). Subcortical Structures and Pathways Involved in Convulsive Seizure Generation. *Journal of Clinical Neurophysiology*.;9:264-277.

Gale, M.K.(1992). GABA and epilepsy basic concepts from preclinical research. *Epilepsia*; 33 Suppl: S3-12.

Ghasi, S., Egwuibe, C., Achukwu, P.U. and Onyeanusi, J.C. (2011). Assessment of the medical benefit in the folkloric use of *Bryophyllum Pinnatum* leaf among the Igbos of Nigeria for the treatment of hypertension. *African Journal of Pharmacy and Pharmacology* 5(1): 83-92.

Ghez, C. and Fahn, S. (1985). "The cerebellum". In Kandel ER, Schwartz JH. *Principles of Neural Science, 2nd edition*. New York: Elsevier. pp. 502–522.

Gill, L.S. (1992). Ethno-medical uses of plants in Nigeria, University of Benin Press, Benin City, Nigeria, pp.46-100.

Gritti, I., Mainville, L., Mancia, M. and Jones, B. E. (1997). GABAergic and other noncholinergic basal forebrain neurons, together with cholinergic neurons, project to the mesocortex and isocortex in the rat. *Jounal of Comparative Neurology*. 383, 163–177.

Guillemain, J., and Tetau, M. (1980). Contribution a 'I'etude d'un "tranquillisant vegetal" Tilia Tomentosa Bourgeons. *cahirers de Biotherapie*, 68: 1-8.

Guilleminault, C. (1989). "Narcolepsy syndrome," in *Principles and Practice of Sleep Medicine,* (edit. Kryger, M.H., Roth, T., Dement, W.C.), W.B. Saunders and Co., Philadelphia PA pp. 338-346.

Guyton, A.C. and Hall, J.E. (2006). Textbook of Medical Physiology, 11th Ed. Elsevier Saunders, Philadelphia. Pp 739.

Haas, H. and Panula, P. (2003). The role of histamine and the tuberomamillary nucleus in the nervous system. *Nature Reviews of Neuroscience*. 4, 121–130.

Haken, H, Kelso, J.A. and Bunz, H. (1985). A theoretical model of phase transitions in human hand movements. Biol Cybern. 51(5):347-56.

Domkin, D., Laczko, J., Jaric, S., Johansson, H., and Latash, M.L. (2002). Structure of joint variability in bimanual pointing tasks. *Experimental Brain Research*. 143(1):11-23.

Henny, P. and Jones, B. E. (2006). Vesicular glutamate (VGlut), GABA (VGAT), and acetylcholine (VACht) transporters in basal forebrain axon terminals innervating the lateral hypothalamus. *Journal of Comparative Neurology*. 496, 453–467.

Hevers, W. and Lüddens, H. (1998). "The diversity of $GABA_A$ receptors. Pharmacological and electrophysiological properties of $GABA_A$ channel subtypes". *Molecular Neurobiology* 18 (1): 35–86.

Hobson, J.A. and Pace-Schott, E. F. (2002). The cognitive neuroscience of sleep: neuronal systems, consciousness and learning. *Nature Reviews Neuroscience*. 679-693.

Husain, G.M., Deepa, M., Singh, P.N., and Vikas, K. (2007). *Ethnopharmacological review of Native Traditional Medicinal Plants for Brain disorders*. *Pharmacognosy Reviews* 1: pp 19-29.

Iber, C., Ancoli-Israel, S., Chesson, A.L., Quan, S.F. (2007). The American Academy of Sleep Medicine. Manual for the Scoring of Sleep and Associated Events: Rules, Terminology and Technical Specifications. Westchester, *American Academy of sleep Medicine*.

International League Against Epilepsy (1989). Proposal for revised classification of epilepsies and epileptic syndromes. Commission on classification and Terminology of the International League Against Epilepsy. *Epilepsia*, 30 (4): 389–399.

International League Against Epilepsy (1993). Guidelines for Epidemiologic Studies on Epilepsy. Commission on Epidemiology and Prognosis, International League Against Epilepsy. *Epilepsia*, 34 (4): 592–596.

Iwu, M.M. (1993). Handbook of African Medicinal Plants. CRC press, Boca Raton, Ann, Arbor and London, Tokyo. Pp 135-136.

Jeannerod, M., M. A. Arbib, G. Rizzolatti, and H. Sakata (1995). Grasping objects: the cortical mechanisms of visuomotor transformation. *Trends in Neuroscience*. 18: 314–320,

Johansson, R. S. and K. Cole. (1993).Sensory-motor coordination during grasping and manipulative actions. *Current Opinions in. Neurobiology.* 2: 815–823,.

Johnston, G.A.R. (2005). $GABA_A$ Receptor channel pharmacology. *Current pharmacological Design*, 11: 1867-1885.

Jones, B. E. (1991). Paradoxical sleep and its chemical/ structural substrates in the brain. *Neuroscience* 40, 637–656.

Jones, B. E. (2004). Activity, modulation and role of basal forebrain cholinergic neurons innervating the cerebral cortex. *Progress in Brain Research*. 145, 157–169.

Jones, B. E. (2005). From waking to sleeping: neuronal and chemical substrates. *Trends in Pharmacological Sciences.* 26, 578–586.

Jones, B. E. and Cuello, A. C. (1989). Afferents to the basal forebrain cholinergic cell area from pontomesencephalic– catecholamine, serotonin, and acetylcholine–neurons. *Neuroscience* 31, 37 –61.

Jones, B.E. (1989). "Basic mechanisms of sleep-wake states," in *Principles and Practice of Sleep Medicine,* (edit., Kryger, M.H., Roth, T., Clem ent, W.C.), W.B. Saunders and Co., Philadelphia PA pp. 121- 140.

Kandel, E. R., Schwartz, J. H. and Jessell, T.M. (2000). *Principles of Neural Science Fourth Edition*. United State of America: McGraw-Hill. p. 324.

Kingsley, R.E. (2000). *Concise Text of Neuroscience* (2nd ed.). Lippincott Williams and Wilkins. Pp 763

Kirtikar K.R. and Basu B. D. (2000). *Indian Medicinal Plants*. 2nd Ed. Allahabad :Oriental Enterprises : pp 1511.

Kostin, A., Kalinchuk, A.V., Stenberg, D. and Porkka- Heiskanen, T. (2006). The inhibition of basal forebrain neurons during sleep deprivation. *Journal of Sleep Research*.15 (S1) Abstracts of the ESRS Congress, Innsbruck.

Koutroumanidis, M., Koepp, M.J., Richardson, M.P., Camfield, C., Agathonikou, A. and Ried, S. (1998). "The variants of reading epilepsy. A clinical and video-EEG study of 17 patients with reading-induced seizures". *Brain* 121 (8): 1409–1427.

Krall, R. L., Penry, J. K., Kuperberg, H.J. and Swinyard, E.A. (1978). *Epilepsia*, , 19, 409-428.

Kryger, M.H., Roth, I., and Dement, W.C., (2005). The brain as a dream state of sex: An activation- synthesis hypothesis of the dream process. *American Journal of Psychiatry*, 134: 1325–1338

Kumar, S.V., Sankar, P. and Varatharajan, R. (2009). Anti-inflammatory activity of roots of *Achyranthes Aspera*. *Pharmaceutical Biology*. 47(10): 973–975

Kuzniecky, R. (1987). "Benign occipital epilepsy: a family study". *Epilepsia* 24: 346–350.

Lader, M. (2008). "Effectiveness of benzodiazepines: Do they work or not?". *Expert Review of Neurotherapeutics* 8 (8): 1189–91.

Lader, M., Tylee, A., and Donoghue, J. (2009). "Withdrawing benzodiazepines in primary care". *CNS Drugs* 23 (1): 19–34.

Lee,M.G., Hassani,O. K.,Alonso,A. and Jones, B. E. (2005). Cholinergic basal forebrain neurons burst with theta during waking and paradoxical sleep. *Journal of Neuroscience.* 25, 4365–4369.

Lewis, E.M. (2001). Should we be concerned about herbal remedies. *Journal of Ethnopharmacology*. 75: 141-164

Loiseau, P., Duche, B., and Cordova, S. (1988). "Prognosis of benign childhood epilepsy with centro-temporal spikes. A follow-up of 168 patients". *Epilepsia* 29 (3): 229–235.

Lokesh J. S., Jagadish V. K., and Harikinan H. (2009). Neuropharmacological evaluation of ethanolic extract of leaves of *Bryophllum pinnatum. Journal of Pharmacy Research.* 2 (5) 811-814.

Lopez, J. I. and Pomposo-Gaztel, Í. (2011). "Patología quirúrgica de la epilepsia". Hospital Universitario de Cruces. http://www.neurologia.com/pdf/Web/5010/bd100616.pdf. Retrieved 24 October .

Lorke, D. (1983). *A new Approach to Acute Toxicity Testing*. *Archives of toxicology*, 54: 275-287

Losier, B.J. and Semba, K. (1993). Dual projections of single cholinergic and aminergic brainstem neurons to the thalamus and basal forebrain in the rat. *Brain Research*. 604, 41 –52.

Mahowald, M. W., Kader, G., and Schenck, C. H. (1997) Clinical categories of sleep disorders *I. Continuum* 3(4): 35–65.

Mahowald, M.W. and Schenk, C.H. (1989). "REM sleep behavior disorder" in *Principles and Practice of Sleep Medicine,* (edit., Kryger, M.H., Roth, T., Dement, W.C.), W.B. Saunders and Co. pp. 389-401.

Marriage, P.B. and Wilson, D.G. (1971). Analysis of the organic acids of Bryophyllum calycinum. *Canadian Journal of Biochemistry*. 49: 282 – 95

McGinty, D. and Szymusiak, R. (2001). Brain structures and mechanisms involved in the generation of NREMsleep: focus on the preoptic hypothalamus. *Sleep Medicine Reviews*. 5, 323–342.

McGinty, D. J. and Harper, R. M. (1976). Dorsal raphe neurons: depression of firing during sleep in cats. *Brain Research*. 101, 569–575.

McIntosh, A., Semple, D., Smyth, R., Burns, J., and Darjee, R. (2005). "Depressants". *Oxford Handbook of Psychiatry* (1st ed.). Oxford University Press. p. 540.

McNamara, J.O. (1989) *Epilepsia*, 30: 13-18.

Mehta, B.J.U. (1952). Studies on Indian medical plant II; bryophyllin, a new antibacterial sustance from the leaves of Bryophyllum, Calyciunum Salsib. *Journal of University Bombay* 21:21-25

Meldrum, B.S., Rogawski, M.A. (2007). "Molecular targets for antiepileptic drug development". *Neurotherapeutics* 4 (1): 18–61

Meldrum, B.S. (1981). GABA agonists as antiepileptic agents. Advanve *Biochemistry of Psycopharmacology*.; 26: 207-17.

Mesulam,M.M., Mufson, E. J.,Wainer, B.H. and Levey,A. I. (1983). Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1–Ch6). *Neuroscience* 10, 1185–1201.

Miriam, H.M and Jennifer, A.K. (2005). "Sodium channel mutations in epilepsy and other neurological disorders". *Journal of Clinical Investigation* 115 (8): 2010–2017.

Miya, T.S., H.G.L. Holck, G.K.W. Yu, and G .R Spratto (1973). Laboratory guide in pharmacology, Burgess Publishing Company, Minneapolis MN, pp: 44-46.

Misra, S., Dixit, S.N. (1979). Anitfungal activity of leaf extract of some higher plants. *Acta Botanica Indica*; 7:147-150

Monnier, M., Dudler, L., Gachter, R. and Schoenenberger, G.A. (1977). "Delta sleep inducing peptide (DSIP): EEG and motor activity in rabbits following intravenous administration," *Neuroscience Letters.* 6(9),

Moore, R. Y. and Bloom, F. E. (1979). Central catecholamine neuron systems: anatomy and physiology of the norepinephrine and epinephrine systems. *Annual Review of Neuroscience*. 2, 113–168.

Morrison, A. A. (1993). Symposium:Dream research methodology: mechanisms underlying oneiric behaviour released in REM sleep by pontine lesions in cats. *Journal of Sleep Research*. 2, 4–7.

Moruzzi, G. and Magoun, H. W. (1949). Brain stem reticular formation and activation of the EEG. *Clinical Neurophysiology*. 1, 455–473.

Musa, A.M., Yaro, A.H., and Danjuma, N.M. (2006). Preliminary phytochemical screening and central nervous system depressant activity of the stem bark of Ficus thoningii blums. *Biol. Environ. Sci. Trop. J.*, 3: 1-6.

Narimatsu, E., Niiya, T., Kawamata, M., and Namiki, A. (2006). "[The mechanisms of depression by benzodiazepines, barbiturates and propofol of excitatory synaptic transmissions mediated by adenosine neuromodulation]" (in Japanese). *Masui* 55 (6): 684–91.

National Institute of Mental Health Retrieved September 3, 2008.

Nauta, W. J. H (1946). Hypothalamic regulation of sleep in rats. An experimental study. *Journal of Neurophysiology*. 9, 285–316.

Nwabuisi, C. (2002). Prohylactic effect of multi-herbal induced in mice. *East African Medical Journal.* 79(7): 343-346

Nwaiwu,J.I., and Akali,P.A.(1986). Anticonvulsant activity of the volatile oil from the fruit of *Tetrapleura tetraptera*. *Journal of Ethnopharmacology*, 18: 103-107.

Oades, R. D. and Halliday, G. M. (1987). Ventral tegmental (A10) system: neurobiology. 1. Anatomy and connectivity. *Brain Research*. 434, 117–165.

Ofokansi K.C., Esimone C.O. and Anele C.K. (2005). Evaluation of the in Vitro combined anti bacterial effects of the leaf extracts of *Bryophyllum pinnatum*.(Fam: crassulaceae) and *Ocimum gratissium* (Fam: Labiate). *Plant Production Research Journal*. 9: 23-27.

Ojewole, J.A.O.(2002). Antihypertensive properties of Bryophyllum pinnatum leaf extracts. *American Journal of Hypertension*; 15(4); A34-A39.

Ojewole, J.A.O. (2005). Antinociceptive, anti-inflammatory and antidiabetic effects of *Bryophyllum pinnatum* (Crassulaceae) leaf aqueous extract. *Journal of Ethnopharmacology*. 99:13–19

Oliver-Bever B. (1983). Medicinal plants in trpical west Africa. III Antinfection therapy with higer plants. *Journal of Ethnopharmacology*, 9: 1 - 83

Olkkola, K.T., and Ahonen, J. (2008). "Midazolam and other benzodiazepines". *Handb Exp Pharmacol*. Handbook of Experimental Pharmacology 182 (182): 335–60.

Olsen, R.W., and Betz, H. (2006). "GABA and glycine". In Siegel GJ, Albers RW, Brady S, Price DD (eds.). *Basic Neurochemistry: Molecular, Cellular and Medical Aspects* (7th ed.). Elsevier. pp. 291–302.

Pace-Schott, E. F. and Hobson J. A. (2002). The neurobiology of sleep: genetics, cellular physiology and subcortical networks. *Nature Review of Neuroscience*.:591-605.

Page, C., Michael, C., Sutter, M., Walker, M., and Hoffman, B.B. (2002). *Integrated Pharmacology* (2nd ed.). C.V. Mosby.

Pal, S. and Nag, C.A.K. (1992). Further studies on the anti-inflammatory profile of the methanolic fraction of the fresh leaf extract of Bryophyllum pinnatum. *Fitoterapia* 63:451-459.

Pal, S. and Nag, A.K. (1989). Preliminary studies on the anti-inflammatory and analgesic activities of Bryophyllum pinnatum (Lam). *Medical Science Research*; 17:561-562

Pal, S. and Nag, A.K. (1991). Studies on the antitulcer activity of a Bryphyllum pinnatum leaf extract in experimental animals. *Journal of Ethnopharmacology*; 33:97-102.

Pal, S., Sen, T., and Nag, C.A.K. (1999). Neuropsychopharmacological Profile of the Methanolic fraction of Bryophyllum pinnatum leaf extract. *Journal of Pharmaceutical Pharmacology*;51:313 -8.

Panayiotopolous, C.P. (2000). "Benign childhood epileptic syndromes with occipital spikes: New classification proposed by the ILAE". *Journal of Child Neurology* 15 (8): 548–552.

Panayiotopoulus, C. P., and Koutroumanidis, M. (2005). *The Significance of the syndromic diagnosis of the Epileses*. *National Society for Epilepsy*.

Panula, P., Yang, H. Y. and Costa, E. (1984). Histaminecontaining neurons in the rat hypothalamus. *Proceedings of the National Academy of Science*. USA. 81, 2572–2576.

Paranjpe, P. (2005). Indian Medicinal Plants forgotten Healers. *Chaukhamba Sanskrit Pratisthan*, 2Delhi, 194-195.

Parmeggiani, P.L. and Rabini, G. (1970). "Sleep, and environmental temperature," *Archieves ltaliennes de Biologie,* 108, 369-387.

Perez, G.R.M., Perez, L.J.A., Garcia, D.I.M. and Sossa, M.H. (1998). Neuropharmocological activity of Solanum nigrum fruit. *Journal of Ethnopharmacology*. 62:43 – 48.

Pollack, C.V. (2006). Seizures. In: Marx J, ed. *Rosen's Emergency Medicine: Concepts and Clinical Practice.* 6th ed. Philadelphia, Pa: Mosby Elsevier;:chap 100.

Porter, R.J., and Meldrum, B.S. (2001). Antiseizure drugs. In: Basic and clinical pharmacology. Katzung BG (ed). New York, Lange Medical Books/McGraw-Hill, pp 403-17.

Prinz, P. (1977) "Sleep patterns in the healthy aged: Relationships with intellectual function," J. *Gerontol,* 32,179-186

Prinz, P.N., Peskind, E.R.and Vitaliano, P.P. (1982). "Changes in the sleep and waking EEGs of nondemented and demented elderly subjects," Journal of *American Geriatrics Society,* 30(2), 86-93

Puri, B.K. and Tyrer, P. (1998). "Clinical psychopharmacology". *Sciences Basic to Psychiatry* (2nd ed.). Churchill Livingstone. pp. 155–156.

Rakotonirina, S.V., Ngo Bum, E., Rakotonirina, A and Bopelet, M. (2001). Sedative Properties of the decoction of the rhizome of *Cyperus articularis*. *Fitoerapia*, 72: 22-29.

Ramya K. B. and Thaakur S. (2009). Evaluation of Neuropharmacological effects of *Dischrostachys cinerea* root. *International Journal of Pharmacological Science and Nanotechnology*. 1(4): 367-374

Ranjan, S., Jadon, V.S., Sharma, N., Singh, K., Parcha, V., Gupta, S. and Bhatt, J.P. (2010). Anti-inflammatory and Analgesic Potential of Leaf Extract of *Allium Stracheyi. Journal of Applied Science Research. 6(2): 139-14.*

Rechtschaffen, A., Bergmann, B.M., Everson, C.A., Kushida, C.A. and Gilliland, M.A. (1989) "Sleep deprivation in the rat: X. Integration and discussion of the findings," *Sleep.* 12,68-87.

Rogawski, M.A. and Bazil, C.W. (2008). "New molecular targets for antiepileptic drugs: alpha(2)delta, SV2A, and K(v)7/KCNQ/M potassium channels". *Current Neurology and Neuroscience Reports* 8 (4): 345–52.

Rogers, (1994). "Autoantibodies to glutamate receptor GluR3 in Rasmussen's encephalitis". *Science* 265 (5172): 648–651

Rosenthal, M.S. (1998). Physiology and neurochemistry of Sleep .American Journal Of Pharmaceutical Education.pp 204-206.

Roth, T. and Roehrs, T (2003). Insomnia: Epidemiology, Characteristics and Consequences. *Clinical cornerstone,* 5(3): 5-15.

Rudolph, U. and Möhler, H. (2006). "GABA-based therapeutic approaches: $GABA_A$ receptor subtype functions". *Current Opinions in Pharmacology* 6 (1): 18–23.

Saïas, T. and Gallarda, T. (2008). "[Paradoxical aggressive reactions to benzodiazepine use: a review]" (in French). *Encephale* 34 (4): 330–6.

Sakurai, T., Amemiya, A., Ishii, M., Matsuzaki, I., Chemelli, R. M., Tanaka, H., Williams, S. C., Richardson, J. A., Kozlowski, G. P., Wilson, S. (1998). Orexins and orexin receptors: a family of hypothalamic neuropeptides and G-protein-coupled receptors that regulate feeding behavior.n *Cell* . 92, 573–585.

Sakurai, T., Nagata, R., Yamanaka, A., Kawamura, H., Tsujino,N., Muraki,Y.,Kageyama, H.,Kunita, S., Takahashi, S., Goto, K. (2005). Input of orexin/hypocretin neurons revealed by a genetically encoded tracer in mice. *Neuron* 46, 297–308.

Salahdeen, H.M. and Yemitan, O.K. (2006). Neuropharmacololgical effects of aqueous leaf extract of *Bryophyllum pinnatum* in mice *African Journal of Biomedical Research.* 9: 101 – 107.

Saper, C. B., Cano, G. and Scammell, T. E. (2005). Homeostatic, circadian, and emotional regulation of sleep. *Journal of Comparative Neurology*. 493, 92–98.

Saper, C.B., Scammel T.E., and Lu, J. (2005). Hypothalamic regulation of sleep and circadian rhythms. *Nature*, 27: 437.

Scholz, J.P. and Schöner, G. (1999).The uncontrolled manifold concept: identifying control variables for a functional task. *Experimental Brain Research*. 126(3):289-306.

Schulz, H. (2008). "Rethinking sleep analysis. Comment on the AASM Manual for the Scoring of Sleep and Associated Events" *American Academy of Sleep Medicine* . 4 (2): 99–103.

Seligman, M.E.P., Walker, E.F. & Rosenhan, D.L..*Abnormal psychology*, (4th ed.) New York: W.W. Norton & Company, Inc.

Seaforth, C.E., Adams C.D., and Sylvester Y. (1983). A guide to the medicinal plants of Trinidad and Tobago. The commonwealth Secretariat, London.

Shafer, Patricia Osborne; Dilorio, Colleen. "Self-Management in Epilepsy Care: Putting Teen and Families in the Center--Part One". Exceptional Parent.

Sherin, J. E., Elmquist, J. K., Torrealba, F. and Saper, C. B. (1998). Innervation of histaminergic tuberomammillary neurons by GABAergic and galaninergic neurons in the ventrolateral preoptic nucleus of the rat. *Journal of Neuroscience*. 18, 4705–4721.

Shorter, E. (2005). "Benzodiazepines". *A Historical Dictionary of Psychiatry*. Oxford University Press. pp. 41–2.

Shporvan, S.D.(1990). Epidemiology, classification, natural history and genetics of epilepsy. *Lancet*. 93:336.

Siegel, J. (2004). Brain mechanisms that control sleep and waking. *Naturwissenschaften*. 91, 355–365.

Siegel, J. M. (2004). Hypocretin (orexin): role in normal behavior and neuropathology. *Annual Review of Psychology*. 55, 125–148.

Simon, D. S. (2004). *The treatment of epilepsy* (2nd ed.). Malden, Mass.: Blackwell Pub.

Soulimani, R., C. Younos, S. Jarmouni-Idrissi, D. BousiaF. Khalouki and A. Laila (2001). Behavioral and pharmaco-toxicological study of Papaver rhoeas L. in mice. Journal of Ethnopharmacology. 74: 265-274.

Sridharan, R. (2002). Epidemiology of epilepsy. *Current Science*, 82 (6): 664-670

Stanley, J.L., Lincoln, R.J.,Brown, T.A., McDonald, L.M., Dawson, G.R. and Reynolds, D.S.(2005). The mouse beam walking assay offers more sensitivity over the rotarod in determining motor coordination deficits induced by benzodiazepines. *Psychopharmacology*., 19 (3): 221-227.

Steininger, T. L., Gong, H., McGinty, D. and Szymusiak, R. (2001). Subregional organization of preoptic area/anterior hypothalamic projections to arousal-related monoaminergic cell groups. *Journal of Comparative Neurology*. 429, 638–653.

Stenberg, D. (2007). Neuroanatomy and neurochemistry of sleep, *Cellular and Molecular Life Sciences* 64. 1187– 1204.

Steriade, M. (2005). Sleep, epilepsy and thalamic reticular inhibitory neurons. *Trends in Neuroscience.* 28, 317–324.

Steriade, M. and McCarley, R. (2005). Brain Control of Wakefulness and Sleep, Kluwer/Plenum, New York. Springer.

Steriade, M. and Timofeev, I. (2003). Neuronal plasticity in thalamocortical networks during sleep and waking oscillations. *Neuron* 37, 563–576.

Steriade,M., Datta, S., Pare,D., Oakson,G. and Curro Dossi, R. C. (1990). Neuronal activities in brain-stem cholinergic nuclei related to tonic activation processes in thalamocortical systems. *Journal of. Neuroscience*. 10, 2541–2559.

Swinnen, S.P., Vangheluwe, S., Wagemans, J., Coxon, J.P., Goble, D.J., Van, A., Sunaert, S., Peeters, R., and Wenderoth, N. (2009). Shared neural resources between left and right interlimb coordination skills: The neural substrate of abstract motor representations. Neuroimage 49(3): 2570-2580

Swinyard, E.A., Woodhead, J.H., White, H.S. and Franklin, M.R. (1989). General Principles: *Experimental selection, quantification, and evaluation of anticonvulsants*. *In*:Levy, R.H., Mattson, B., Melrum, J.K. and Dreifuss, F.E. (Eds) *Antiepileptic Drugs,* 3rd edition. Raven Press New York, pp 85-103.

Szymusiak, R. and Satinoff, D.(1981). "Maximal REM sleep time defines a narrower thermoneutral zone than does minimal metabolic rate," *Physiology and Behavior.* 26,687-690

The National Society for Epilepsy (2009), *What is Epilepsy?*.

Timmann, D. and Daum, I. (2007). "Cerebellar contributions to cognitive functions: a progress report after two decades of research". *Cerebellum* 6 (3): 159–62.

Tosini G., Pozdeyev N., Sakamoto K. (2008). The circadian clock system in the mammalian retina. *Bioessays*, 30 (7): 624-633.

Trease, G.E. and M.C. Evans, (1983). Textbook of Pharmacognosy. 13th Edn., Bailliere, Tindall, London, pp: 683-684.

Trost, L.F., Wender, R.C., Suter, C.C., Von Worley, A.M., Brixner, D.I., Rosenberg, J.H. and Gunter, M.J (2005). "Management of epilepsy in adults. Treatment guidelines". *Postgraduate Medicine* 118 (6): 29–33.

Trulson, M. E. (1985). Activity of dopamine-containing substantia nigra neurons in freely moving cats. *Neuroscience and Biobehavioral Reviews*. 9, 283–297.

Trulson, M. E. and Preussler, D. W. (1984). Dopaminecontaining ventral tegmental area neurons in freely moving cats: activity during the sleep-waking cycle and effects of stress. *Experimental Neurololgy*. 83, 367–377.

Turner, T.H.; Drummond, S.P.A.; Salamat, J.S.; Brown, G.G. (2007). Effects of 42 hr sleep deprivation on component processes of verbal working memory [Electronic version]. *Neuropsychology, 21*, 787–795.

Tyrer, P. (1989). Risk of dependence on benzodiazepine drugs: The importance of patient selection. *British Medical Journal*, 298: 102-105.

Vanni-Mercier,G., Gigout, S.,Debilly,G. and Lin, J. S. (2003). Waking selective neurons in the posterior hypothalamus and their response to histamine H3-receptor ligands: an electrophysiological study in freely moving cats. *Behavioral Brain Research*. 144, 227–241.

Viswanatha, S.A.H.M., Thippeswamy, A.H.M., Manjala, D.V., Meahendra K.C.B. (2006). Some neuropharmacological effects of the methanolic root extract of Cissus quadrangularis in mice. *African Journal of Biomedical Research*. 9: 69-75.

Vogel, G.W., Thurmond, A., Gibbons, P., Sloan, K., Boyd, M. and Walker, M., (1975). "REM sleep reduction effects on depression syndromes," *Archieves of General Psychiatry,* 32,765-776.

von Economo, C. (1923). Encephalitis lethargica.*Wien Med Wochenschr*. 73, 777–782.

von Economo, C. (1930). Sleep as a problem of localization. *Journal of Nervous and Mental Diseases*. 71, 248–259.

Wakeel, O.K., Aziba, P., Ashorobi, R.B., Umukoro, S., Aderibigbe, A.O. and Awe, E.O. (2004). Neuropharmacological activities of ficus platyphylla stem bark in mice. *African Journal of Biomedical Research*. 5 -78.

Walker, M.P. (2009)."The Role of Sleep in Cognition and Emotion." Annals of the New York Academy of Sciences. 1156.: 174.

Weiss, P. and Jeannerod, M. (1998). Getting a Grasp on Coordination. *News in Physiological Sciences,* 13: 70-75

Weiss, P., Y. Paulignan, M. Jeannerod, and H. J. Freund (1997). Quantitative analysis of complex movement: temporal characteristics. *Experimental Brain Research Supplement.* 117: S55,.

Westmoreland, B.F., Benarroch, E.E., Dube, J.R., Regan, T.J. and Sandok, B.A. (1994). *Medical neurosciences.* Mayo Foundation Rochester, pp 307-12.

White, H.S., Wolf, H.H., Woodhead, J.H. and Kupferberg, H.J. (1998). The National Institute of Health anticonvulsant drug development program: Screening for efficacy. *In*: French, J., Leppik, I.E. and Dichter, M.A. (Eds.) *Antiepileptic Drug Development: Advances in Neurology*, Vol. 76, Lippincott-Raven Publishers Philadelphia, 29-39.

World Health Organization (1996): *WHO Guideline for the Assessment of herbal Medicines: WHO expert committee on specification for pharmaceutical preparation*. Technical Report series No 863. Geneva.

World Health Organization (2001). *Epilepsy: Aetiology, Epidemiology and Prognosis*. Fact sheet No 165.

Wilson, J., and Reynolds, E. (1990). Translation and analysis of a cuneiform text forming part of a Babylonian treatise on epilepsy. *Medical History*, 34 (2): 185–98.

Wolf,U., Rapoport, M.J., and Schweizer, T.A. (2009). "Evaluating the affective component of the cerebellar cognitive affective syndrome". *Journal of Neuropsychiatry and Clinical Neuroscience.* 21 (3): 245–53.

Won, S.W., Kuk, H.S., Sam, S.K. (1980). Chemistry and pharmacology of flavones-C glycosides from Ziziphus seeds. *Korean Journal of Pharmacology.* 11: 141-148.

Wong, W. (1976). Some folk medicinal plants from Trinidad, *Econ, Bot.,* 30:103-142.

Woolf, N. J. (1991). Cholinergic systems in mammalian brain and spinal cord. *Progress in. Neurobiology*. 37, 475–524.

Yoshida, K., McCormack, S., Espana, R. A., Crocker, A. And Scammell, T. E. (2006). Afferents to the orexin neurons of the rat brain. *Journal of Comparative Neurology* 494, 845–861.

Zavala, F. (1997). "Benzodiazepines, anxiety and immunity". *Pharmacology and Therapeutics.* 75 (3): 199–216.

Zisterer, D.M. and Williams, D.C. (1997). "Peripheral-type benzodiazepine receptors". *General Pharmacology.* 29 (3): 305–14.

APPENDICES

Appendix I: Effect of hydro-methanol leaf extract of *Bryophyllum pinnatum* on motor coordination (Beam walk assay) in mice.

Treatment / Dose		Number of foot slips	Latency (s)
Normal saline	1ml/kg	0.40 ± 0.24	14.20 ± 2.65
Diazepam	1.5mg/kg	9.80 ± 1.62*	50.60 ±2.64*
Extract	25mg/kg	7.20 ± 0.66*	38.40 ± 6.28*
Extract	50mg/kg	7.0 ±1.00*	57.80 ± 2.20*
Extract	100mg/kg	3.0 ± 0.95	31.20 ± 9.16

Values are given as mean ± SEM; experimental groups were compared with control. Values are statistically significant at * = $p < 0.05$,

Appendix II: Effect of hydro-methanol leaf extract of *Bryophyllum pinnatum* on exploratory behaviour (Hole board) in mice.

Treatment / Dose		Number of head dips in 5 minutes
Control	1ml/kg	15.60 ± 1.81
Diazepam	1.5mg/kg	10.20 ±3.80
Extract	25mg/kg	8.80 ± 2.60
Extract	50mg/kg	14.80 ± 3.84
Extract	100mg/kg	11.00 ± 2.68

Values are given as Mean ± SEM; experimental groups were compared with control. Values are statistically significant at *= $p< 0.05$,

Appendix III: Effect of hydro-methanol leaf extract of *Bryophyllum pinnatum* on diazepam-induced sleep in mice

Treatment / Dose		Onset of sleep (min)	Duration of sleep (min)
Control	1ml/kg	3.60 ± 0.68	11.80 ± 1.71
Extract	25mg/kg	3.00 ± 1.05	218.20 ± 60.24*
Extract	50mg/kg	2.60 ± 0.24	269.60 ± 53.07*
Extract	100mg/kg	2.8 ± 0.37	298.20 ± 54.37*

Values are given as mean ± SEM; experimental groups were compared with control. Values are statistically significant at *= $p < 0.05$,

Appendix IV: Effect of hydro-methanol leaf extract of *Bryophyllum pinnatum* on PTZ-induced convulsion in mice.

Treatment / Dose	Onset of clonic convulsion	Protection (%)	Mortality (%)
Control 1ml/kg	6.80 ± 2.74	0.0	100
Valproic acid 200mg/kg	0.00 ± 0.00	100	0.0
Extract 25mg/kg	19.00 ± 1.00	80	20
Extract 50mg/kg	6.75 ± 2.43	20	80
Extract 100mg/kg	12.00 ± 3.89	60	40

Values are given as mean ± SEM; experimental groups were compared with control. Values are statistically significant at *=$p < 0.05$