Evaluation of sedative & hypnotic activities of *Thysanolaena maxima by* Hole board method

A Dissertation submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

Submitted By

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Declaration by the Research Candidate

Habiba Ahmed, ID: 2012-3-70-024, hereby declare that the dissertation entitled "Evaluation of sedative and hypnotic activities of *Thysanolaena maxima* by hole board method*"* submitted by me to the Department of Pharmacy, East West University and in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, under the supervision and guidance of **Nazia Hoque**, Senior Lecturer, Department of Pharmacy, East West University, Dhaka.

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Dedication

This Research Paper is dedicated to

My beloved parents,

Who are my biggest inspirations…

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Abstract

Anxiety affects one-eighth of the total population worldwide and has become an important area of research in psychopharmacology during this decade. Benzodiazepines (BZDs) are the major class of compounds used in anxiety and they remain the most commonly prescribed treatment for anxiety. However, the realization that BZDs have a narrow safety margin has prompted many researchers to evaluate new compounds in the hope of identifying other anxiolytic drugs with fewer unwanted side effects. The plant *Thysanolaena maxima* has been used by the natives in Tao Dam Forest, Bangkok and *Khashi* traditional healers and village folks in Meghalaya. It is used as a traditional medicine for the treatment of cancer, In case of Red eye and Dirty, in treatment of Dysentery, to facilitate Delivery, in veterinary medicine and as mouth wash in fever. The aim of the present study was to evaluate the anxiolytic activity of methanol extract of *Thysanolaena maxima* by hole-board method. The hole board test is useful for modeling anxiety in animals, in this test an anxiolytic-like state may be reflected by an increase in head dipping behaviors. Groups of mice were treated orally with CMC (1g/100ml), diazepam (1 mg/kg), and *T. maxima* (200mg/kg & 400 mg/kg). Evaluations were done in 0 minutes and then 30 minutes post-treatment and the duration of observation was mostly 5 min. In the hole-board test, mice were observed for number/duration of head dips and number of head poke. A significant increase in the exploratory head-dipping behavior was observed after treatment with 200mg/kg and 400 mg/kg of *Thysanolaena maxima* extract, thus reinforcing the hypothesis that it has anxiolytic-like activity. Based on the results of the present study, it can be concluded that that all the extracts of *Thysanolaena maxima* possesses potent anxiolytic or CNS depressant properties, which support its use in traditional medicine. However, further studies are needed to understand the exact mechanisms of action and to isolate the compound (s) responsible for such activity.

Key Words: *Thysanolaena maxima,* Anxiolytic, Hole board, CNS depressant.

Chapter 1

Introduction

1.1 Overview on Medicinal Plants

Fossil Records has revealed the use of medicinal plants by human beings around 60,000 years ago during Middle Paleolithic Age (Fabricant & Farnsworth, 2001). These Fossil records suggest that even Neanderthal were not an exception who did not make use of medicinal plants (Das & Choudhury, 2012). Example of such medicinal plants is *Gingko biloba* which has been used medicinally for thousands of years. It is used for the treatment of numerous conditions such, many of which are under scientific investigation. The species has an evolutionary lineage that dates back to the Lower Jurassic, about 190 million years ago. Although this genus has undergone much change over this length of time, fossilized leaf material from the Tertiary species Ginkgo adiantoides is considered similar or even identical to that produced by modern *Ginkgo biloba* trees (Jalalpour *et al.,* 2012).

Gingko biloba, the Gingko plant is wide used in Alzheimer's disease, Cerebrovascular Diabetes, Insufficiency, Cognitive Enhancement, Depression, Intermittent Claudication, Macular Depression, PMS, Sexual Dysfunction, Tinnitus (Pelton, 2000).

The Plant kingdom consists of many different plant species containing different substances of medicinal importance. Some of these have already been explored for biological activity while some are not (Rahman *et al.,* 2008). As a source of medicine plant materials are important components of health care system. There are about 250,000 higher plant species (both Angiosperms and Gymnosperms) with a lower limit of 215,000 and upper limit of 500,000. Among these only 6% have been screened for biological activity and 15% have been evaluated phytochemically (Fabricant & Farnsworth, 2001). Only just in South East Asia and its surrounding parts, there exist about 50,000 plant species among which 3,000 plants have been documented for potential medicinal properties and around 6,000 plants are used by traditional practitioners (Shariff *et al.,* 2006). So, Plants have been the traditional source of raw materials for medicine. It is known through the scholastic works of Atharva Veda and the writings of Charaka and Sushruta which gave huge knowledge of preventive and curative medicinal to the scientific community (Chowdhury *et al.,* 2008). Now, nearly 95% of plants used in traditional medicines are collected from forests and other natural sources. The plants collected from different sources show wide disparity in therapeutic values and also much variation in market rates (Maridass & De Britto, 2008). It has been estimated that about

13000 plant species around the world are used as drugs. Since, the inclination of using natural product has increased; the exploration of active plant extracts has become frequent for new drug discovery (Ferdous, Imam & Ahmed, 2010). Over 50% of all advanced clinical drugs are made of natural products that play an important role in drug development programs of the pharmaceutical industry. There are hundreds of medicinal plants which have a long history of curative properties against various diseases. However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the higher plant (Razvy, Faruk & Hoque, 2011). So, for being cheap, relatively safe and easily available, medicinal plants and herbs embody the foundation of traditional medicinal practice all over the world. Representing an untapped and huge reservoir of drugs either known or novel in origin, the medicinal plants are center of research to find out novel lead compounds (Ambikar *et al.*, 2010).

1.2 Medicinal Plants

The term of medicinal plants include a various types of plants used in herbalism and some of these plants have a medicinal activities. These medicinal plants consider as a rich resources of ingredients which can be used in drug development and synthesis. Besides that these plants play a critical role in the development of human cultures around the whole world. Moreover, some plants consider as important source of nutrition and as a result of that these plants recommended for their therapeutic values. These plants include ginger, green tea, walnuts and some others plants. Other plants their derivatives consider as important source for active ingredients which are used in aspirin and toothpaste. These days the term “Alternative Medicine” became very common in western culture, it focus on the idea of using the plants for medicinal purpose. But the current belief that medicines which come in capsules or pills are the only medicines that we can trust and use. Even so most of these pills and capsules we take and use during our daily life came from plants. Medicinal plants frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and antimalaria medications, contain ingredients from plants. Moreover the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, respectively.

1.3 Future of Medicinal Plants

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies. Characteristics of Medicinal Plants Medicinal plants have many characteristics when used as a treatment, as follow:

• Synergic medicine- The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

• Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

• Preventive medicine- It has been proven that the component of the plants also characterize by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment (Rasool Hassan, 2012).

1.4 Goals of Using Medicinal Plants as Therapeutic Agents

The goals of using plants as sources of therapeutic agents are –

a) To isolate bioactive compounds for direct use as drugs, (E.g. Digoxin, Digitoxin, Morphine, Reserpine, Taxol, Vinblastine, Vincristine);

b) To produce bioactive compounds of novel or known origin as lead compounds for semi synthesis to produce molecules of higher activity and / or lower toxicity, (E.g. Metformin, Nabilone, Oxycodone and other narcotic analgesics, Taxotere, Teniposide, Verapamil, and Amiodarone, which are based on Galegine, Δ9 – tetrahydrocannabinol, Morphine, Taxol, Podophyllotoxin, Khellin respectively);

c) To use agents as pharmacologic tools (E.g. LSD, Mescaline, Yohimbine); and

d) To use the whole plant or part of it as a herbal remedy, (E.g. Cranberry, Echinacea, Feverfew, Garlic, *Ginkgo biloba*).

1.5 Approaches to Drug Discovery Using Higher Plants

In the past, plant extracts were evaluated mainly in experimental animals, primarily mice and rats. The most extensive of these programs were sponsored by the National Cancer Institute (NCI) in the United States and the Central Drug Research Institute (CDRI) in India. More than 35,000 species were screened in vitro and later in vivo at NCI from 1960

to 1981. Taxol and camptothecin were discovered in this program as well as several other plant-derived compounds that were unsuccessful in human studies. In 1986 the NCI program abandoned this approach and continued to collect and screen plants using a battery of 60 human tumor cell lines and also initiated a screening of plants for anti-HIV activity in vitro. Calanolide A, currently in Phase I clinical trials, was developed from this program. The CDRI evaluated approximately 2,000 plant species for several biologic activities,including antibacterial, antidiabetic, antifertility, antifungal, antihypercholesteremic, anti-inflammatory, antitumor, cardiovascular, central nervous-system depressant, cytotoxicity, diuretic, and others. To date no biologically active drugs for human use have arisen from that program, even though a large number of known and novel bioactive compounds were isolated from the active plants. Several types of ethnomedical information are available: Plants used in organized traditional medical systems. Ayurveda, Unani, Kampo, and traditional Chinese medicine have flourished as systems of medicine in use for thousands of years. Their individual arrangements all emphasize education based on an established, frequently revised body of written knowledge and theory. These systems are still in place today because of their organizational strengths, and they focus primarily on multicomponent mixtures. The value of plants used in traditional medicine for drug discovery as lacking credibility; undeniably they are used widely by most people on this planet. Adverse effects from those widely used plants are not well documented in the literature, and efficacy of these plants and plant mixtures is more difficult to assess by Western scientific methods (Fabricant & Fransworth, 2001).

1.6 The Central Nervous System

The organ of the central nervous system that is likely most familiar to us, yet still holds the greatest mysteries for physiologists, is the brain. Enclosed completely by the skull, the brain is composed primarily of nervous tissue. This remarkable organ consists of about 100 billion cells called neurons, or nerve cells that enable everything from the regulation of breathing and the processing of algebra to performing in the creative arts. At the foramen magnum, the brain merges with the next organ of the central nervous system: the spinal cord. The spinal cord passes through the vertebral foramen of the first cervical vertebra and continues inferiorly to the first or second lumbar vertebra. It contains fewer cells than the brain, with only about 100 million neurons. The spinal cord enables the

brain to communicate with most parts of the body below the head and neck; it is also able to carry out certain functions on its own.

1.6.1 Neurons

The billions of neurons in nervous tissue are directly responsible for its sensory, integrative, and motor functions. Neurons are the excitable cell type responsible for sending and receiving signals in the form of action potentials. Recall that most neurons are *amitotic,* meaning that at a certain point in development, they lose their centrioles and after that lack the ability to undergo mitosis. Luckily, neurons are very long-lived cells, and some can easily survive the entire lifespan of an organism if given adequate nutrition and oxygen in a supportive environment.

Neurons vary greatly in size. Some tiny neurons in the CNS are only 1 mm long, whereas some PNS neurons may be up to 1 m or longer. As shows, most neurons consist of three parts: the central cell body, where the majority of the biosynthetic processes of the cell occur; one or more dendrites, which carry electrical signals to the cell body; and one axon, the long "arm" that generally carries electrical signals away from the cell body.

1.6.2 The Cell Body

The most conspicuous part of a neuron is its large cell body**,** or soma, which ranges from 5 to 100 μm in diameter. The cell body is the most metabolically active part of the neuron, because it is responsible for maintaining the sometimes huge cytoplasmic volume of the neuron and also for manufacturing all of the proteins the neuron needs. This high level of biosynthetic activity is reflected in the composition of the organelles within its cytoplasm:

- Free ribosome and rough endoplasmic reticulum (RER) are found in abundance, reflecting the commitment of the cell body to protein synthesis.
- Other organelles involved in protein synthesis, including the Golgi apparatus and one or more prominent nucleoli, are present.
- Mitochondria are found in large numbers, indicating the high metabolic demands of the neuron.

Additionally, the cytoplasm of the cell body contains lysosomes, smooth ER, and other organelles found in most cells. The characteristic shape of the cell body is maintained by another component of the cytoplasm—the neuronal cytoskeleton, which is composed of

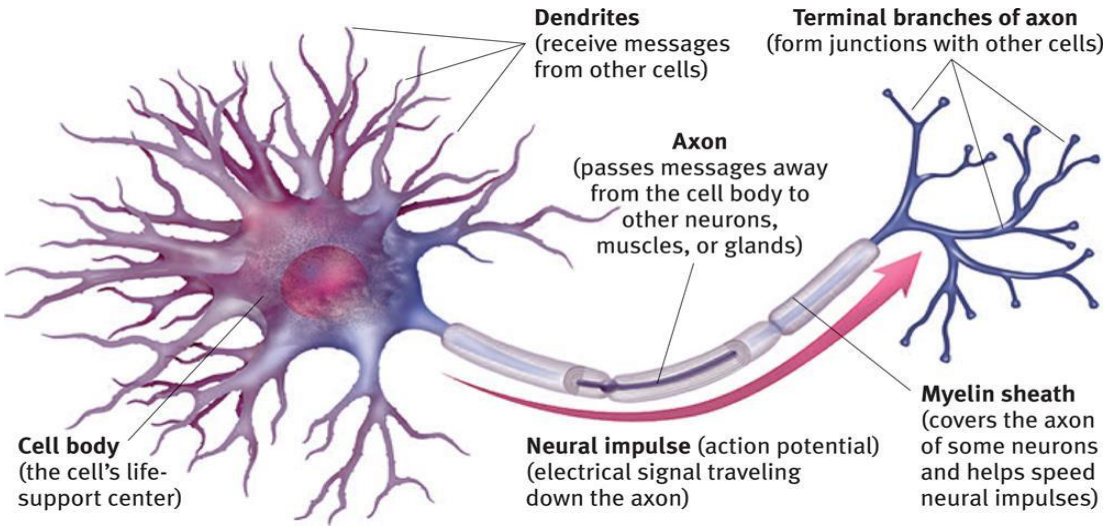

Fig 1.1: Simple diagram of neuron

intermediate filaments. These filaments bundle together to form larger structures called neurofibrils, which provide structural support that extends out into the dendrites and axon of the neuron as well. The cytoskeleton also contains microtubules that provide structural support and a means for transporting chemicals between the cell body and the axon.

1.6.3 Processes: Dendrite and axon

Extending from all neuron cell bodies are long "arms," cytoplasmic extensions that are called processes. These processes allow the neuron to communicate with other cells. Most neurons have two types of processes, including one or more dendrites and one axon.

1.6.3.1 Dendrites

Dendrites are typically short, highly forked processes that resemble the branches of a tree limb. They receive input from other neurons, which they transmit in the form of electrical impulses toward the cell body. Note, however, that dendrites usually do not generate or conduct action potentials. Their cytoplasm contains most of the same organelles as the cell body, including mitochondria, ribosomes, and smooth endoplasmic reticulum. The extensively forked "dendritic trees" of most neurons give them a huge receptive surface area. Interestingly, the branches of the dendritic tree change throughout an individual's lifetime: They grow and are "pruned" as a person grows and develops and as functional demands on the nervous system change.

1.6.3.2 Axon

Although a neuron may have multiple dendrites, each neuron has only a single axon**,** sometimes called a nerve fiber. Traditionally, an axon was defined as a process that carried a signal away from the cell body. However, the axons of certain neurons can carry a signal both toward and away from the cell body. For this reason, new criteria have been developed to define an axon: They are considered processes that can generate and conduct action potentials. Depending on the type of neuron, the axon may range in length from short to very long; in some neurons the axon accounts for most of the length of the neuron. For example, the axons of motor neurons going to the foot must extend from the lumbar portion of the spinal cord all the way down the lower limb and to the foot.

1.6.4 Neuronal synapse

A synapse is where a neuron meets its target cell. Neuronal synapses generally occur between an axon and another part of a neuron; they may occur between an axon and a dendrite, an axon and a cell body, and an axon and another axon. These types are called axodendritic, axosomatic, and axoaxonic synapses.

Regardless of the type of synapse, we use certain terms to describe the neurons sending and receiving the message:

Presynaptic neuron: The presynaptic neuron is the neuron that is sending the message from its axon terminal.

Postsynaptic neuron: The postsynaptic neuron is the neuron that is receiving the message from its dendrite, cell body, or axon. The transfer of chemical (neurotransmitters) or electrical signals between neurons at a synapse is called synaptic transmission**,** and it is the fundamental process for most functions of the nervous system.

1.6.5 Neurotransmitters

They are made in either the cell body or the axon terminal and packaged into synaptic vesicles, they are released from the presynaptic neuron, they bind to their receptors on the postsynaptic membrane, and finally their effects are often rapidly terminated through removal and/or degradation.

Neurotransmitter Receptors: Two types of neurotransmitter receptors have been identified:

Ionotropic receptors: They directly control the movement of ions into or out of the neuron when bound by a neurotransmitter.

Metabotropic receptors: They are directly connected to metabolic processes that begin when they are bound by neurotransmitters.

1.6.5.1 Major Neurotransmitters

Acetylcholine

The best-studied and one of the most widely used neurotransmitters by the nervous system overall, is the small-molecule neurotransmitter acetylcholine (ACh).

The Biogenic Amines

also called the *monoamines* are a class of five neurotransmitters synthesized from amino acids. Three of the biogenic amines form a subgroup called the catecholamines, all of which are synthesized from the amino acid tyrosine.

Norepinephrine

It influences functions such as heart rate, blood pressure, and digestion. Neurons that secrete norepinephrine in the CNS are largely confined to the brainstem, where they work to regulate the sleep/wake cycle, attention, and feeding behaviors.

Epinephrine

It has the same effects as norepinephrine.

Dopamine

It helps to coordinate movement, and is also involved in emotion and motivation. The receptor for dopamine in the brain is a target for certain illegal drugs, such as cocaine and amphetamine, and is likely responsible for the behavioral changes seen with addiction to these drugs. Another biogenic amine is serotonin, which is synthesized from the amino acid tryptophan. Most neurons that use serotonin are found in the brainstem, and their axons project to multiple places in the brain (Amerman, 2016).

Amino Acids

The amino acids of primary interest to the pharmacologist fall into two categories: the neutral amino acids glycine and **GABA** and the acidic amino acid glutamate. All of these compounds are present in high concentrations in the CNS and are extremely potent modifiers of neuronal excitability.

GABA

GABA receptors are divided into two types: GABA-A and GABA-B. GABA-A receptors open chloride channels and are antagonized by picrotoxin and bicuculline, which both cause generalized convulsions. GABA-B receptors, which can be selectively activated by the antispastic drug baclofen, are coupled to G proteins that either inhibit calcium channels or activate potassium channels. In most regions of the brain, IPSPs (inhibitory postsynaptic potential) have a fast and slow component mediated by GABA-A and GABA-B receptors, respectively. Immunohistochemical studies indicate that a large majority of the local circuit neurons synthesize GABA. A special class of local circuit neuron localized in the dorsal horn of the spinal cord also synthesizes GABA. These neurons form axoaxonic synapses with primary sensory nerve terminals and are responsible for presynaptic inhibition.

1.7 Basic Pharmacology of Sedative-Hypnotics

An effective sedative (anxiolytic) agent should reduce anxiety and exert a calming effect. The degree of central nervous system depression caused by a sedative should be the minimum consistent with therapeutic efficacy. A hypnotic drug should produce drowsiness and encourage the onset and maintenance of a state of sleep. Hypnotic effects involve more pronounced depression of the central nervous system than sedation, and this can be achieved with most drugs in this class simply by increasing the dose. Graded dose-dependent depression of central nervous system function is a characteristic of sedative-hypnotics. However, individual drugs differ in the relationship between the dose and the degree of central nervous system depression. The linear slope for drug A is typical of many of the older sedative-hypnotics, including the barbiturates and alcohols. With such drugs, an increase in dose above that needed for hypnosis may lead to a state of general anesthesia. At still higher doses, sedative-hypnotics may depress respiratory and vasomotor centers in the medulla, leading to coma and death. Deviations from a linear

dose-response relationship, as shown for drug B, will require proportionately greater dosage increments in order to achieve central nervous system depression more profound than hypnosis. This appears to be the case for benzodiazepines and certain newer hypnotics; the greater margin of safety this offers is an important reason for their widespread use to treat anxiety states and sleep disorders.

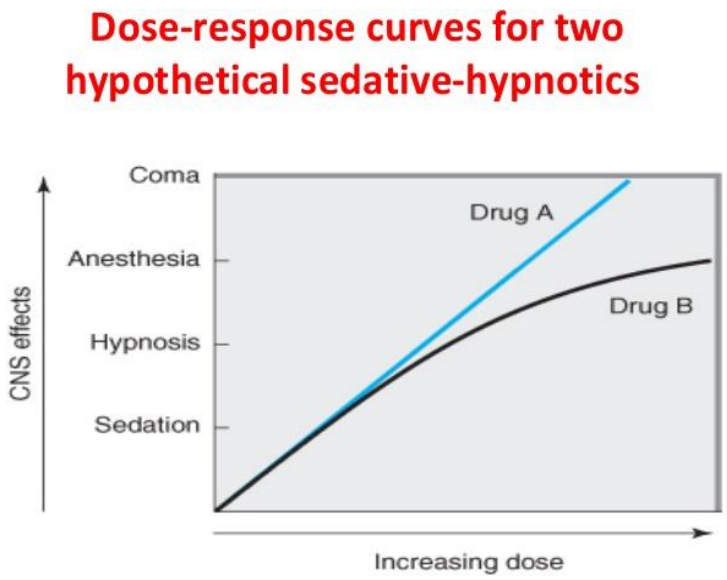

Fig 1.2: Dose response curve for two hypothetical sedative hypnotics

1.8 Benzodiazepine (BZD)

BZDs act as positive allosteric modulators on the gamma amino butyric acid GABA-A receptor. The GABA-A receptor is a ligand-gated chloride-selective ion channel.

GABA is the most common neurotransmitter in the central nervous system, found in high concentrations in the cortex and limbic system. GABA is inhibitory in nature and thus reduces the excitability of neurons. GABA produces a calming effect on the brain. The 3 GABA receptors are designated A, B, and C.

The GABA-A receptor complex is composed of 5 glycoprotein subunits, each with multiple isoforms. GABA-A receptors contain 2 α subunits, 2 β subunits, and 1 γ subunit. Each receptor complex has 2 GABA-binding sites but only 1 BZD-binding site. The benzodiazepine binding site is in a specific pocket at the pairing (intersection) of α and γ subunits. Within the α subunit of isoforms 1, 2, 3, and 5 resides a histidine residue (H101, H101, H126, and H105, respectively) that possesses a high affinity for BZDs. Isoforms 4 and 6 of the α subunit contain an arginine residue and do not have an affinity for BZDs.

BZDs bind to the pocket created by the α and γ subunits and induce a conformational change in the GABA-A receptor, allowing GABA to bind. BZDs bind to the pocket created by α and γ subunits and induce a conformational change in the GABA-A receptor. This alteration, in turn, induces a conformational change in the GABA-A receptor's chloride channel that hyperpolarizes the cell and accounts for GABA's inhibitory effect throughout the central nervous system (Griffin, 2013).

1.9 Mechanism of Action of Benzodiazepine

BZD act selectively on GABA-A receptors, which mediate inhibitory synaptic transmission throughout the central nervous system. BZD enhance the response to GABA by facilitating the opening of GABA activated chloride channels. They bind specifically to a regulatory site on the receptor, distinct from GABA binding sites, and act allosterically to increase the affinity of GABA for the receptor. Single channel recordings show an increase in the frequency of channel opening by a given concentration of GABA, but no change in the conductance or mean open time, consistent with an effect on GABA binding rather then the channel-getting mechanism. BZD do not affect receptors for other amino acids, such as glycine or glutamate (Rang *et al.*, 2007).

1.9.1 Indication of Benzodiazepine

1. Sleep disorder
2. Anxiety disorder
3. Seizure disorder
4. Alcohol detoxification
5. Muscle relaxant.

1.9.2 Classification of Sedative Hypnotics

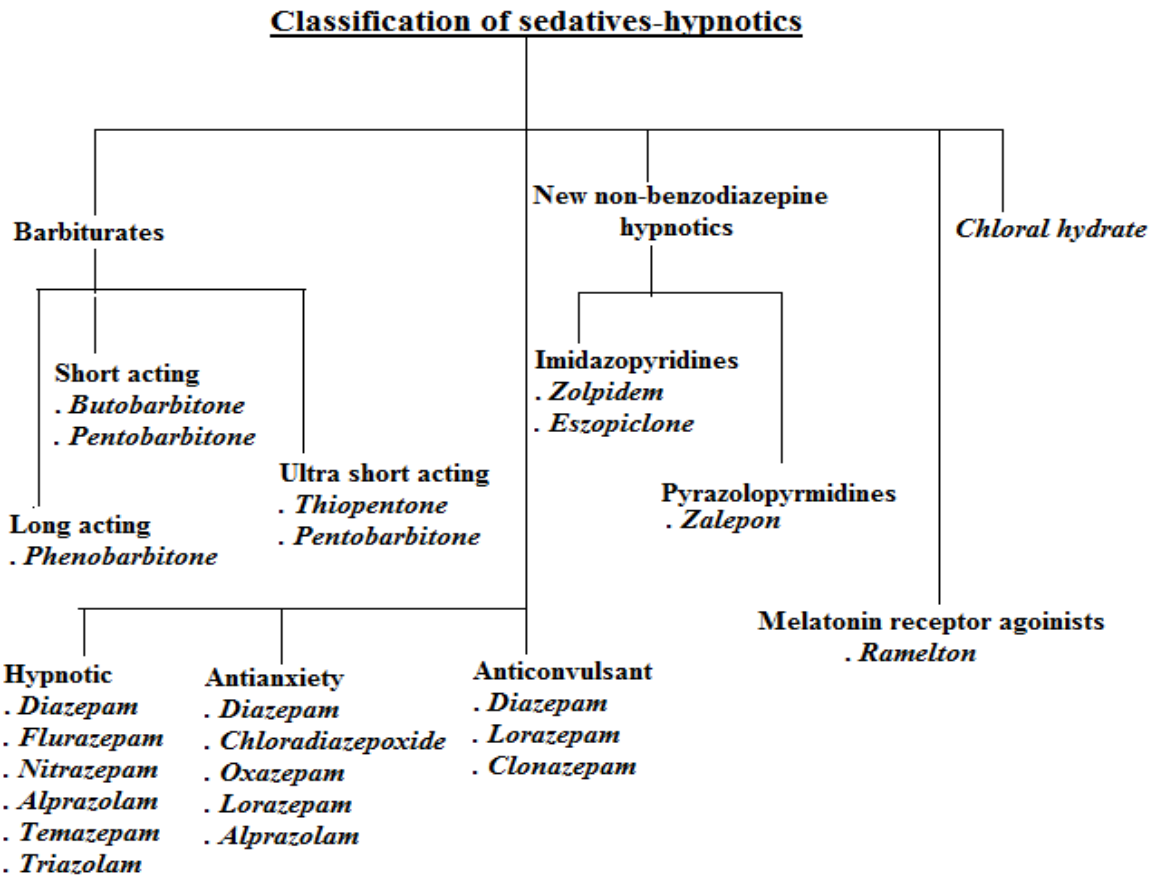

Fig 1.3: Classification of sedatives-hypnotics

1.9.3 Diazepam

Diazepam is a long-acting, medium-potency BZD that is used as an anticonvulsant and for anxiolysis, sedation, and myorelaxation. Diazepam, one of the most common BZDs used for anxiety, is available in intramuscular, intravenous, oral, and rectal gel forms. Diazepam interacts with equal affinity on all BZD-sensitive receptors in the central nervous system. Anxiolytic effects are seen at low doses because of diazepam's interaction with α2-containing receptors in the limbic system. At higher doses, diazepam may provide myorelaxation in addition to anxiolysis; the myorelaxant effect is primarily mediated through α2-containing receptors in the spinal cord and motor neurons and to a lesser extent. Through interaction with α3-containing receptors. Of course, at higher doses, sedation and anterograde amnesia are also noted, but these effects are α1-mediated. Diazepam is unique in that its metabolism in the liver produces the active metabolites oxazepam, temazepam, and desmethyldiazepam, each of which exerts its own

action. These metabolites and their actions account for diazepam's long elimination half-life, which increases approximately 1 hour for each year of age over 40 (e.g., the diazepam elimination half-life in a 75-year-old would be approximately 75 hours). Thus, when prescribing this drug, clinicians must consider potential side effects related to active metabolite buildup, such as over sedation and anterograde amnesia. These side effects can be serious and long-lasting, especially in the elderly and in those with hepatic or renal dysfunction. For intravenous administration, diazepam must be prepared in solution with propylene glycol to be water soluble; this solution can cause pain on injection and, in some cases, thrombophlebitis (Griffin, 2013).

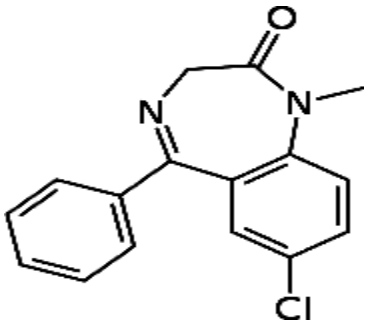

Fig 1.4: Structure of Diazepam

1.10 CNS Disorders

1. Confusion

Confusion is a cognitive disorder characterized by loss of the normal coherent stream of thought or action. The three variations of confusion are agitated delirium, somnolence, and incoherence.

2. Coma

Coma is a state of eyes-closed unresponsiveness in which even the most vigorous stimulation fails to arouse the patient. Clues to the etiology of coma obtained from the history include the presence of trauma, evidence of intoxication, and history of cardiac, pulmonary, hepatic, and renal disease.

3. Aphasia

Aphasia is an acquired disorder of language resulting from brain damage.

4. Dementia

Dementia is the chronic and progressive loss of memory and at least one other cognitive

function (language, praxis, object knowledge, or executive function) which interferes with a person's ability to perform their activities of daily living.

5. Visual Loss and Other Visual Disturbances

Most patients with visual loss have ophthalmologic problems such as cataracts, glaucoma, and macular degeneration. With some exceptions such as angle closure glaucoma and retinal detachment, most of these conditions tend to develop slowly over months to years.

6. Diplopia

Patients with a variety of neurologic, ophthalmologic, and psychiatric disorders complain of diplopia.

7. Disorders of the Eyelids and Pupils

8. Facial Weakness, Dysarthria and Dysphagia

9. Dizziness and Vertigo

10. Proximal and Generalized Weakness

11. Focal Limb Weakness

12. Rapidly Progressive Weakness

13. Parkinsonism

Parkinsonism refers to the combination of bradykinesia (slowness of movement) and rigidity. It is the core feature of Parkinson disease and other disorders of the extrapyramidal system such as progressive supranuclear palsy, multisystem atrophy, and corticobasal degeneration.

13. Hyperkinetic Movement Disorders

Although classically associated with basal ganglia dysfunction, hyperkinetic movement disorders may be due to pathology at one of several levels of the central nervous system, and in some cases, the peripheral nervous system.

14. Tremor

Tremor is the rhythmic oscillation of a body part caused by alternating contraction of agonist and antagonist muscles. Patients describe tremor as shaking or trembling, or may specifically use the word tremor.

15. Distal and Generalized Sensory Symptoms

Pain is the sensory symptom which most frequently brings a patient to neurologic attention.

16. Back Pain, Myelopathy, and Radiculopathy

17. Gait Disorders

18. Seizures and Epilepsy

An epileptic seizure is a transient occurrence of neurologic signs and/or symptoms due to abnormal, excessive, synchronous neuronal activity in the brain.

19. Stroke

Stroke is a sudden-onset neurologic syndrome caused by infarction or hemorrhage within the central nervous system

20. Multiple Sclerosis

21. Intracranial Mass Lesions (Tarulli, 2016).

1.11 CNS Activity in Natural Origin

Depression is an important health care problem in the world that is characterized by several signs such as intense sadness, despair, and recurrent thoughts of death or suicide. Although several synthetic drugs are available for treatment of depression, side effects such as dry mouth, hypotension, fatigue, sexual dysfunction and drowsiness limit the use of these treatments .Therefore, researches for new antidepressant drugs with fewer side effects are needed.

Insomnia, defined as persistent difficulty in falling or staying asleep that affects daytime function, can induce significant psychological and physical disorders. Most patients engage in long-term use of benzodiazepines analogs to treat insomnia. But these drugs have limited benefits with obvious side-effects, such as impaired cognitive function, memory and general daytime performance .In addition, long-term administration results in tolerance and dependence. Numerous herbal medicines are recognized as active in the central nervous system (CNS) and they have at least a hypothetical potential to affect chronic conditions such as anxiety, depression, headaches or epilepsy, that do not respond well to conventional treatments. People from different regions of the world have used herbal medicines to alleviate affective disorders for many years and as a consequence, the search for novel pharmacotherapy from medicinal plants has progressed significantly in the past decade .An increasing number of herbal products have been introduced into psychiatric practice (Mirshafa, Azadbakht & Ahanger, 2013).

1.12 Sedative and Anxiolytic Activities of Various Phytochemicals from Plant

Terpenoids

Many essential oils and monoterpenes are used therapeutically as relaxing drugs and traquilizers. Themonoterpenes are present in volatile oils of many plant species such as *Mentha piperita, Zanthoxyum schinifolium* and *Mentha X villosa*. The inhalation of the crude extract of *Kaempferia galanga* L showed sedative effects at doses of 1.5 mg. The essential oils Linalool. 1, 8-cineole [14] and α-terpineol were shown to possess sedative properties showed that the bitter sesquiterpene lactones, Lactucin and Lactucopicrin which were isolated from *Lactuca virosa* and *Cichorium intybus* exhibited sedativeproperties in the spontaneous locomotor activity test on mice. Galphimine B, a nor-secotriterpenoidfrom *Galphimia glauca* (cav.) Kuntze (Malpighiaceae) was shown to exhibit a strong depressant activity on the nervous system. A-gurjuene, benzyacetone and (+)- Calarene which are the main constituents of Agarwood oil (*Aquilaria sinensis*) and Spikenard (*Nardostachys jatamansi*) exhibited sedative activity when tested on mice; but it was noted that the most effective dose of the compounds was lower than their original content in the oil (α-Gurjuene (1.5%), Calarene (0.17%), Benzylacetone (0.1%). A preliminary study of the sedative effect of monoterpene alcohols in mice led to the isolation ofisopulegol, neoisopulegol, (±)-Isopinocampheol, (-)- myrtenol, (-)-cis-myrtenol, (+)-p-menth-1-en-9-ol and (±)-neomenthol. These compounds exhibited a depressant effect in pentobarbital-induced sleep test, indicating a sedative property. It was reported that Linalyl acetate isolated from *Lavandula angustifolia* Mill possess sedative activity. Caryphyllone and linalool volatile oils obtained from *Milissa officinalis* L. were shown to be responsible for the plants sedative properties. The resins humulone, 2-methyl-3-butane-2-ol and lupulone which are the main ingredients found in *Humuluslupulus* have been shown to possess sedative action. Investigation of the seed oil of jujube seed (*Zizyphus vulgaris*) showed that it possesses sedative properties.

Flavonoids

Albizzia julibrissin Durazz flowers are used as sedatives in oriental traditional medicine. The phytochemical study of this plant led to the isolation of two flavonol glycosides, quercetrin and isoquercetrin. These compounds were observed to increase pentobarbital-induced sleeping time in adose-dependent manner in mice. Binding assays conducted on

two CNS inhibitorytargets: benzodiazepine and GABA (A) receptors showed that Luteolin-7-diglucoronide isolated from *Lippia alba* possess sedative properties with half maximal inhibitory concentration (IC50 = 101 and 40 micron respectively). The flavonoids Swertisin, Spinosin, 6'''-sinapoylspinosin, 6''' feruloylspinosin and p-coumaroylspinosin isolated from the seeds of *Zizyphus vulgaris* Lamark var. spinosus Bunge (Rhamnaceae) were identified as some of the active principles responsible for its sedative properties. The seeds of *Ziziphus jujuba* Mill var. spinosa (Bunge) Huex. H. F. Chou are used as sedatives in China. Out of the eight flavonoids isolated from it only two of them (spinosin and Swertish) were shown to possess significant sedative activity. It was believed by some authors that the spinosin isolated from *Zizyphus. jujuba* is responsible for the sedative activity of the plant. It was reported by Marder *et al.,* (2003) that the flavonoid2S (-)-hesperidin isolated from *Valeriana officinalis* has sedative and sleep enhancing properties whereas 6-methylapigenin alsoisolated from *Veleriana officinalis* exhibited ability to increase the sleep enhancing properties of hesperidin. The sedative activity of the butanol fraction was attributed to the presence of flavonoids which constitute the major part of the butanol fraction. Apigenin isolated from *Chamomilla recutita* (L) Rausch was shown to possess a mild sedative effect and a clear anxiolytic activity.

Alkaloids

Sanjoinine-A and Nuciferine are alkaloids isolated from Sanjoin (*Zizyphus vulgaris*) showed strong sedative activity. Sanjoinine-A was observed to be very potent at a dose of 3 mg/Kg. In order to determine if there was any additivity, synergistic or counteracting interaction between the alkaloids isolated from the plant; the potent alkaloids were co-administered with the non-potent alkaloids. Coclaurine and sanjoinine-A were co-administered with nuciferine and sanjoinine-A with coclaurine. It was observed that there is additivity between sanjoinine-A and nuciferine, while coclaurine did not enhance the sedative activity of sanjoinine-A. The sedative activity of the alkaloid was monitored by measuring the hexibarbital induced sleeping time. It was also postulated that since the major constituent of the butanol fraction obtained was zizyphusine, a quartinary aporphine alkaloid which did not exhibit any sedative activity; the sedative activity of the butanol fraction was attributed to the presence of flavonoids or the minor components such as aporphine alkaloids (caeverine, N-methylasimilobine and Norisocorydine). It was

shown that the compounds atropine, hyoscyamine and scopolamine induce sleep and abolish the awaking effect. These compounds isolated from *Atropa bell-donna* L and *Datura stramonium* L.

Steroids

Two bufadienolides known as diagremontianin and bersaldegenin-1, 3, 5-orthoacetate isolated from *Kalanchoe diagremontiana* were shown to have a pronounced sedative effect. Aquirre-observed that β-sitisterol isolated from *Tillia americana* var, Mexicana exhibit sedative activity at a dose of 30 mg/Kg in mice. A dose response curve of β-sitisterol in the range of 1-30 mg/kg doses indicated that this compound produces an anxiolytic-like action from 1-10 mg/kg and a sedative response when the dose was increased to 30 mg/kg. These effects were observed to resemble those produced by diazepam (0.1 mg/kg).

Saponins

The saponins jujuboside A and B isolated from the seeds of *Zizyphus vulgaris* Lamark var. spinosus Bunge (Rhamnaceae) was thought to be partly responsible for the sedative property of the plant observed that jujuboside A had no inhibitory activity but exerts a synergism with phenylalanine on the central nervous system function, therefore concluded that jujuboside A is not a sedative agent. But Jiang *et al.*, (2007) showed that saponins extracted from the species *Ziziphus jujuba* Semen that grows in China exhibited significant effect on walking time and coordinated movement, and prolonged the suprathreshold barbiturate induced sleeping time.

Quinoids

Ternstroemia pringlei is used in Mexico for the treatment of insomnia. Bioactivity guided fractionation of the methanolic extract led to the isolation of the sedative compound jacaranone which is a quinoid. It gave a dose-dependent response of ED50 = 25 mg/Kg mouse weight (Rahman *et al.*, 2013).

1.13 Research on Herbal Drug

Herbal drug may be defined as the plants, plant parts and plant products of all description, particularly those with medicinal properties. Herbal drugs are generally manufactured by the combination of two or more natural substances. The utility of these combinations are:

1. To increase efficacy of the drug.
2. To remove toxic effects.
3. To reduce side-effects.
4. To maintain stability.
5. To keep pleasant taste, color and odor.

1.14 Aims and Objectives

1.14.1 Aims

There is a continuous and urgent need to discover new medicinal compounds with diverse chemical structures and novel mechanisms of action for new and re-emerging infectious diseases. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against the harmful diseases. The increasing failure of chemotherapeutics, severe adverse effects with increase doses and repeated use of drugs, problems with multiple dosage regimens and antibiotic resistance exhibited by pathogenic microbial infectious agents and emergence of new diseases has led to the screening of medicinal plants throughout the world for their potential activity. Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi-processed plant produce drugs and medicines. Thus huge foreign exchanges can be saved if the manufacturers, to satisfy their needs, utilize the indigenous medicinal plants or their semi processed products. *Thysanolaena maxima* are a medicinal plant used traditionally in Nepal, northern and eastern parts of India, and Bhutan. Upon significant literature survey it was found only a little research work has been performed on this plant to evaluate its medicinal value and active constituents those are responsible for its pharmacological activities. Therefore, taking into consideration the traditional uses of the plant and facilities available for conducting the study, this research work was performed on the plant *Thysanolaena maxima*.

1.14.2 Objectives

In order to achieve these aims, the following research objectives have been identified:

Plant: *Thysanolaena maxima.* (Methanol Extract)

SL No.	Experiment
1.	Pharmacological Activity Test
a.	**Sedative & Hypnotic Activity Study**

1.15 Plant Material

Plants are potent biochemists and have been components of phytomedicine since times Immemorial; man is able to obtain from them a wondrous assortment of industrial chemicals. Plant based natural constituents can be derived from any part of the plant like bark, leaves, flowers, roots, fruits, seeds, etc. i.e. any part of the plant may contain active components. The systematic screening of plant species with the purpose of discovering new bioactive compounds is a routine activity in many laboratories. Scientific analysis of plant components follows a logical pathway. Plants are collected either randomly or by following leads supplied by local healers in geographical areas where the plants are found. Fresh or dried plant materials can be used as a source for the extraction of secondary plant components. Many authors had reported about plant extract preparation from the fresh plant tissues. The logic behind this came from the ethno medicinal use of fresh plant materials among the traditional and tribal people. But as many plants are used in the dry form (or as an aqueous extract) by traditional healers and due to differences in water content within different plant tissues, plants are usually air dried to a constant weight before extraction. Other researchers dry the plants in the oven at about 40°C for 72 hr. In most of the reported works, underground parts (roots, tuber, rhizome, bulb etc.) of a plant were used extensively compared with other above ground parts in search for

bioactive compounds possessing antimicrobial properties (Das, Tiwari & Shrivastava, 2010).

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1.16 Plant Information

Thysanolaena maxima are a perennial grass plant found in hilly regions of Nepal, northern and eastern parts of India, and Bhutan. The flowers of this plant are used as cleaning tool or broom, which is known as “Amriso” in Nepali."Tiger Grass" is a common name for this plant throughout the tropics where it is grown as an ornamental. It may be used to create the effect of bamboo, which it resembles, but to which it is not related. It also is called "broom grass" in areas where its flowers are used as a cleaning too (Quattrocchi, 2014).

Thysanolaena maxima or Tiger Grass has been under-used as a landscape plant, until recent years. It is easily mistaken for a Bamboo as they share a lot of the same traits. Tiger Grass is made up of numerous long slender canes which are topped with drooping, green, bi-lobed leaves. The leaves only grow out of the very top of the canes which gives the plant a mushroom like appearance. When mature, Tiger Grass will start to produce purple flowers which resemble the tassels on corn. The canes don't produce side shoots, so plants maintain a neat and tidy appearance, unlike some Bamboo species.

Fig 1.5: *Thysanolaena maxima*

Another great feature of *Thysanolaena* is that it does not produce runners, which means your Neighbor’s won’t find any popping up in their back yard.

1.17 Scientific Names of Plant

Agrostis maxima Roxb. ***Melica latifolia*** Roxb., ***Thysanolaena agrostis*** Nees

Family: *Poaceae*

Table 1.1: Vernacular Names of *Thysanolaena maxima*

English	Asian Broom Grass, Bamboo grass, bouquet grass, broom grass, tiger grass
India	bushnia, chir, chiten, deobahari, garajono, hmunphiah, jharu, jurna, karsar, konda, phuljharu, pirlu, saper
Indonesia	awis, lantebung, menjalinwuwu
Laos	dokkhein, kheemkhoong
Malaysia	bulohteberau, rumputbuloh
Nepal	Amriso
Philippine	buybuyeagadu, lasa, tagadeu, tagisa
Thailand	khoeilaa, khoei la, laolaeng, toing kong, tong, Kongyakapphaiyai
Tibet	Khrergod
Vietnam	cay le, dong trung ha thao, omganh, say

1.18 Taxonomic Hierarchy of the Investigated Plant

Table 1.2: Taxonomic hierarchy of Thysanolaena maxima

Rank	Scientific Name and Common Name
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta – Vascular plants
Superdivision	Spermatophyta – Seed plants
Division	Magnoliophyta – Flowering plants
Class	Liliopsida – Monocotyledons
Subclass	Commelinidae
Order	Cyperales
Family	Poaceae / Gramineae – Grass family
Genus	*Thysanolaena* Nees – tiger grass
Species	*Thysanolaena maxima* (Roxb.) Kuntze [excluded]

1.19 Growth Pattern of Plant

Broom grass forms tussocks. The culms arise centrifugally during the peak growth period (June–July) and bear inflorescence at the end of vegetative growth. The appearance and growth of culms in a tussock depict a characteristic order that probably controls the extent of culm growth, as well as the size, number, and length of the leaves and the overall shape of the crown. Broom grass is usually planted during April and May, and peak vegetative growth takes place during June and July. The productive period starts with the flowering of the plant in the months of October to March. The inflorescence becomes ready for harvest by December and January and the harvest continues until March. The maximum height of a tussock is attained in three years, while basal girth and culms numbers continue to increase (Tiwari *et al.*, 2012).

1.20 Distribution

Broom grass grows in almost all parts of Meghalaya, where it covers an estimated 127 sq. km (Tiwari *et al.*, 1995). Broom grass grows below 1,600 m.a.s.l. on a wide range of soils. It naturally colonies areas with newly exposed soils due to land slip road sides, abandoned quarries, abandoned jhum (shifting cultivation) areas, and waste lands.

Large areas of abandoned jhum fields have also been converted to broom grass plantations in the last two decades, due to an increase in demand for brooms from various parts of the country. The RiBhoi and East Khasi Hills districts account for more than 70% of the total production of brooms in Meghalaya (Tiwari *et al.*, 2012).

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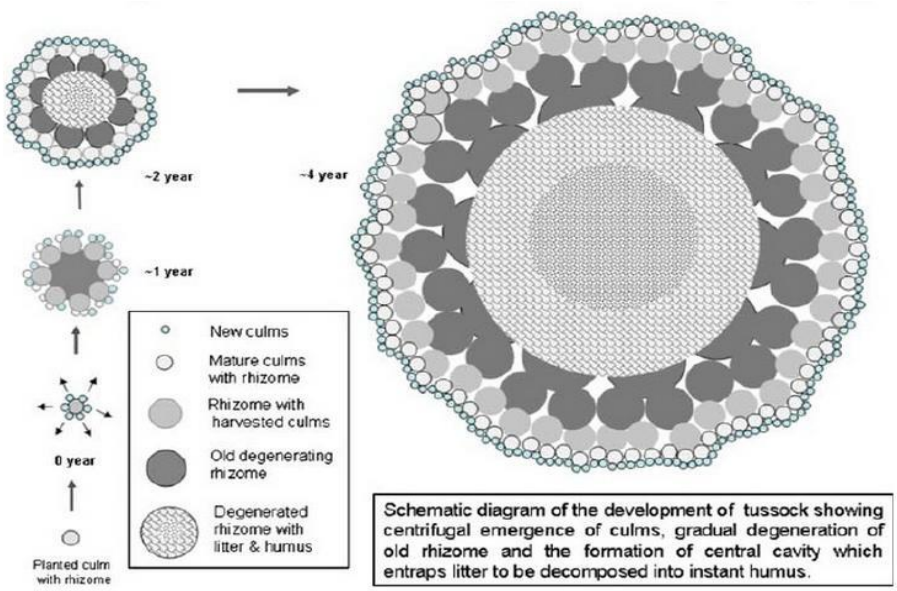

Fig 1.6: Centrifugal emergence and growth of tussock year-wise

1.21 Production and Processing

The quality of broom depends upon the time of harvesting. Shorter inflorescences, generally collected in the early stages of inflorescence development, are considered the best quality. The product is classified under three categories:

(a) Class-(I) or best quality: those types in which the flowers have not yet opened and are collected in the months of January and February.

(b) Class-(II) or medium quality: those types that are cut immediately after flowering and are collected in the months of (late) February and March.

(c) Class-(III) or inferior quality: those types that have remained in the culms for longer periods and are collected in the months of April and May.

After harvesting, the product is transported to homestead for processing, which is usually a simple process. A frame-like structure in the form of trays made of bamboo is used for drying the inflorescence. Sometimes, the inflorescences are tied in small bundles and hanged over fixed bamboo poles. The drying operation is done over three to four days for hardening the stems in order to prevent rotting. The product is then packed in large

bundles and transported to the market or stocked in one place in the villages to sell to middle men or traders. The majority of the product enters the market and is transported to other places at this level of processing only. Value addition, that is, the making of broom,

Fig 1.7: Plant of *Thysanolaena maxima*

is done manually by very few households and for a small quantity of the total harvest. Meghalaya has now emerged as one of the largest producers and exporters of broom grass in the country. Ninety percent of the brooms produced are exported outside the state. There is a trend of an increase in production, price, and growers' income. This may be attributed to the expanding market for the product. The steady increase in the price shows that the price was regulated by external demand. The drop in price during 2005 and 2006 may be attributed to the doubling in the production within a year, possibly causing a glut in the market. However, during the subsequent years, when production either increased moderately or plateaued, the price continued to increase *(*Tiwari *et al.*, 2012).

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1.22 Medicinal Use of *Thysanolaena maxima*

1. Leaf past of *Thysanolaena maxima* with the leaf paste of *Litsea lancifolia* is given in case of Dysentery.

2. Seeds of *Thysanolaena maxima* powdered and given to women before childbirth to facilitate delivery. The flour used as an abortifacient, contraceptive.

3. Inflorescence paste mixed with a pinch of slaked lime is applied locally for treatment of boils or cancer.

4. Young stem juice is applied on the eye when eyes become red and dirty.

5. Vomiting tendency, stomach trouble. Crushed flowers are taken with water.

6. A decoction of 200-300 gm of young roots for one dose is used twice a day in case of bronchial problem. Poultice of young flowers is used in rheumatic pain and skin swelling.

7. The roots are used in flatulence and leaves are traditionally used in performing religious ceremony. Lodhas prescribe a paste of the flowers along with country liquor and honey as contraceptive to women. Decoction of roots is used along with common salt for the remedy of mouth sore (Dutta *et al.,* 2008).

1.23 Study Area

The research was carried out in the Pharmacognosy Lab, Microbiology Lab, Chemistry Lab and Pharmacology Lab of Department of Pharmacy, East West University, Dhaka.

1.24 Data Collection

All the relevant data has been collected from two types of sources:

Primary sources: direct personal contact and observations of the experiments carried out in the laboratory.

Secondary sources: various publications like journals, papers, documents and websites.

1.25 Research Protocol

1. Selection, identification, collection, drying and grinding of plants.
2. Extraction of the powders with methanol and collection of extract.
3. Phytochemical analysis of the plant extract.
4. Sedative and Hypnotic activity determination.
5. Studying and comparing the results obtained.

1.26 Information Processing and Analysis

The data and the results collected were reviewed, compared, processed and organized. Some tests were repeated to be sure of the results. Some data were analyzed into flow charts and statistical tables where possible.

Chapter 2

Literature Review

2.1 Chemical Composition and Nutritive Value of Kuchi (*Thysanolaena maxima*) Grass

In this study, investigated the chemical composition and Nutritive value of *Thysanolaena maxima* which is on DM basis was 15.31 CP, 3.82 EE, 23.58 CF, 47.69 NFE, 9.60 TA, 0.61 Ca and 0.21 P ,12.07 per cent DCP, 50.89 per cent TDN, 3.22 NR (Nutritive ratio), 35.96 per cent SE, 2239Kcal/kg DE and 1832 Kcal/kg ME. (Bhuyan, Das & Baruah, 1988)

2.2 Medicinal Plants in Tao Dam Forest, Wangkrajae Village, SaiYok District, Kanchanaburi Province

This study was investigated for potential medicinal plant resources in Tao Dam Forest. *Thysanolaena maxima* (Roxb.) boiled with *Hyptis capitata* Jacq. And lin-ma (unknown species) for drink/ tonic (Chiramongkolgarn & Paisooksantivatana, 2014).

2.3 Herbal remedies among the Khasi Traditional Healers and village folks in Meghalaya

The study investigated the use of medicinal plant among the Khasi traditional healers. They use the Inflorescence paste of *Thysanolaena maxima* (Roxb.), mixed with a pinch of a slaked lime and applied locally for treatment of boils and cancer. Young stem juice is applied on the eye when eyes become red and dirty (Hynniewta & Kumar, 2008).

2.4 Phytostabilization Potential of Pb Mine Tailings by Two Grass Species, *Thysanolaena maxima* and *Vetiveria zizanioides*

Two grass species, *T. maxima* and *V. zizanioides*, were used to conduct the experiments. Prior to testing, all grasses were acclimatized in the greenhouse for 2– 3 months (temperature, 27–29°C; approximately 70 % relative humidity; 17,568-lx light intensity; and 12/12-h photoperiod). The 100 % survival rates of *T. maxima* and *V.zizanioides* grown on Pb mine tailings and the absence of symptom toxicity indicated both plants' ability to withstand a high contamination of Pb.It appears that *T. maxima*, reported to withstand Pb concentration up to 100,000 mg kg−1 (Rotkittikhun et al. 2006), is similarly suited to grow in a wide range of habitats, including degraded mining areas (Meeinkuirt *et al.*, 2013).

2.5 Growth pattern, production, and marketing *of Thysanolaena maxima* (Roxb.) Kuntze: An important nontimber forest product of Meghalaya, India

The dry weight of stems or leaves per culm did not increase significantly with the age of the tussock and the overall ratio of the leaf to stem weight per tussock during the first and second year of growth was around 1. The ratio, however, increased slightly during the third year and significantly during the fourth year. Total biomass per tussock increased from about half a kg at the end of the first year of growth to about 12 kg at the end of year 4. The average productivity increased up to the third year and decreased drastically beyond the fourth year onward. Farmers, however, still harvest the crops as new culms, arising during the fourth and fifth year of growth, providing some brooms even during thesixth year. The observations were not extended beyond the fourth year due to disarrayed patterns in vigor loss (Tiwari *et al.*, 2012).

2.6 What drives elevational pattern of phytolith diversity in *Thysanolaena maxima* (Roxb.) O. Kuntze A study from the Darjeeling Himalayas

Different climatic and edaphic factors i.e., temperature, rainfall, actual evapotranspiration (AET), potential evapotranspiration (PET), moisture index (MI) and soil pH along a tropical–temperate elevation gradient (150–2456 m a.s.l.) in the Darjeeling Himalayas would influence the variability and plasticity of formation and frequencies of phytoliths in *Thysanolaena maxima*.. Morphometric measurements show a positive correlation between dimensions of stomate, three-lobates and bilobates with MAT, AET, PET and soil pH and a negative correlation with MI. Length of bilobate shanks and dimension of bulliform cells are found to be negatively correlated with MAT, AET, PET and soil pH and positively correlated with MI; however, an opposite trend is noticed for shank width. No significant relationship is observed with rainfall, but MI, which is a measure of the water balance between rainfall and PET of an area shows significant correlation with morphometric traits (Dey, Ghosh & Beraa, 2016).

2.7 Mechanical and Dielectric Properties of *Thysanolaena maxima* (Broom Grass) Long Fibre Reinforced Polyester Composites

The untreated and chemically treated fibre is reinforced into the polyester matrix and the composites are fabricated to test their mechanical and dielectric properties strictly as per ASTM procedures. The highest tensile strength of 82.39 MPa, modulus of 1.05 GPa is obtained for broom grass CT – 1, CT – 2 fibres respectively. With CT – 2, 3 broom grass FRP composites achieved highest tensile strength, modulus respectively at maximum fibre volume fraction. Broom grass CT – 2 FRP composites have shown good flexural strength, modulus of 78.51 MPa, 5.31 GPa respectively than the other composites investigated in the present work. Impact strength of 91.11 kJ/m2 is achieved for broom grass FRP composites at 39.35 % fibre volume fraction. The insulating light weight material according to required dielectric strength is selected from the composites reinforced with broom grass at different volume fractions of fibre (Srinivasababu, Kumar & Reddy, 2014).

2.8 Effect of plant density on growth and yield of *Thysanolaena maxima*: an important non-timber forest product of Meghalaya

Thysanolaena maxima is a wild grass cultivated by the farmers of Meghalaya. When thedemand for broom increased, many erstwhile shifting cultivators got motivated to take up cultivation of this plant. The study revealed that the growth and yield parameters are not impacted by plant density during the first year of its growth. During the second year, the effect of density on growth and yield became pronounced and 1.5x2.0 m spacing gave optimum number of tiller, tiller diameter, internodal length, leaf number, panicles number, harvest index and height and diameter of tussock. The yield of panicles was however maximum in the treatment 1.0x1.0 m spacing. The study concludes that up to two time harvests 1.5x2.0 m spacing may be adopted if farmers are interested for green biomass (fodder). However, for optimum production of broom grass panicles (broom), 1.0x1.0 m spacing is most appropriate (Lapasam & tiwari, 2016).

2.9 Evaluation of highly efficient monomeric sugar yield from Thai Tiger grass (*Thysanolaena maxima*)

The monomeric sugar yield from *T. maxima* by two-stage microwave/chemical

pretreatment and enzymatic hydrolysis is evaluated. The optimal conditions of the pretreatment were investigated by varying reaction times, reaction temperatures and chemical concentrations to maximize the amount of obtained monomeric sugar. The *T. maxima*was treated with microwave-assisted NaOH pretreatment using 15:1 liquid-to-solid ratio (LSR), 1% (w/v) NaOH at 140 °C for 15 min, followed by microwave-assisted H_2SO_4pretreatment using 15:1 LSR, 0.5% (w/v) H_2SO_4 at 200 °C for 5 min. The maximum monomeric sugar released was 30.2 g/100 g of NaOH-pretreated solids. The enzymatic hydrolysis of the microwave-/chemical-pretreated *T. maxima* at pH 4.8, 45 °C for 120 h using enzyme amount of 160 μl/g pretreated solids produced an impressive maximum sugar yield of 110.4 g/100 g of NaOH-pretreated solids (Komolwanicha *et al.*, 2016).

2.10 The chemopreventive effects of *Thysanolaena latifolia* against carbon tetrachloride (CCl4)-induced oxidative stress in rats

Rats were pre-treated with *T.latifolia* prior to the administration of CCl4. Hepatic damage and toxicity were evaluated by measuring the levels of serum transaminases, malondialdehyde content, reduced glutathione, antioxidant enzymatic molecules and histopathological changes in rats. Administration of CCl4 to rats (1.2 ml/kg, p.o.) showed a significant elevation of lipid peroxidation and decreased enzymatic and nonenzymatic antioxidants. Consistent with these changes, CCl4 treatment enhances hepatic damage as evidenced by sharp increases in serum transaminases. Pretreatment of animals with *T.latifolia*(150 and 300 mg/kg, p.o.) showed significant and dose-dependent protection against CCl4-mediated oxidative liver damage and toxicity in rats. Furthermore, histopathology of *T.latifolia* treated animals showed tendency toward normalization of cytoarchitecture of liver. *T.latifolia* was found to possess 20.3 ± 0.72 mg/g total phenolic content expressed as gallic acid equivalent and to scavenge DPPH radical significantly. *T.latifolia* possesses significant hepatoprotective effect against oxidative damage mediated by CCl4 in rats, which may be due to its antioxidant and free radical scavenging effects (Iqbal, Gnanaraj & Haque, 2012).

2.11 Studies of *Thysanolaena maxima* on oral health care

Majority of these plant species are used as natural tooth brush. Certain trees are used for management of gum bleeding, toothache, sores in mouth and bad breath. Stem, Young Twigs, Leaves, Bark, Fruit, Spines, Seeds and latex are the parts of trees being exploited for oral health care. Plant-based traditional knowledge has become a recognized tool in search for new sources of drugs; it is clear that these herbal medicines can offer a platform for further research in dentistry. During the last few decades there has been an increasing interest in the study of medicinal plants and their traditional use in different parts of the world. Present literature documentation reveals that medicinal plants continue to play a major role in oral healthcare needs of Indian population. Hence there is an urgent need to conserve the biodiversity as well as the traditional knowledge by proper documentation and for further research in dentistry (Kumar P, 2014).

Chapter 3

Material and Methods

3.1 Collection & Preparation of Plant Material

Thysanolaena maxima plant was collected in the month of June, 2014 from Chittagong Hill tracts during rainy season when weeds were in their maximum densities. Then proper identification of plant sample was done by an expert taxonomist. The leaves of the plant were sun dried for several days. The plant materials were then oven dried for 24 hours at considerably low temperature for better grinding. The dried leaves was then ground in coarse powder using high capacity grinding machine in the Phytochemical Research Laboratory, Department of Pharmacy, East West University.

3.2 Washing and Drying of *Thysanolaena maxima* Plant

At first the leaves were thoroughly washed with tap water to remove dust, soil, bird's droppings etc. within them. The leaves were dried under sunlight for one week. But, due to rainy season sun drying was avoided. Instead, the leaves were dried in hot air oven at 50°C for 2 hours.

3.3 Grinding and Storage of Dried Samples

The dried parts were ground to coarse powder with the help of home blender machine. This process breaks the plant parts into smaller pieces thus exposing internal tissues and cells to solvents and facilitating their easy penetration into the cells to extract the constituents. Then the powdered sample was kept in clean closed glass containers till extraction. During grinding of sample, the grinder was thoroughly cleaned to avoid contamination with any remnant of previously ground material or other extraneous matters deposited on the grinder. The total weight of the dried powdered leaf was 700 gm which was measured using electronic balance and it was found to be 700 gm.

3.4 Extraction of the Dried Powdered Sample

The fine powder of *T. maxima* leaves was dissolved in 7L methanol and it was thoroughly shaken to dissolve the powder into the solvent. Then it was kept in a closely covered glass jar for 7 days and shaken several times during the process for more interaction between the powdered particles and the solvent. This process is termed as maceration. The cover of the jar was closed properly to resist the entrance of air in the jar.

3.5 Filtration of the Extract

After the extraction process the plant extracts was filtered with sterilized cotton filter and filter paper. The filtrate was collected in a beaker. The filtration process was repeated three times by using cotton and filter paper. Then the filtrate was taken into a volumetric flask and covered with aluminum foil paper was prepared for rotary evaporation.

3.6 Evaporation and Condensation of the leaf extracts

The extracts were transferred to the round bottle flask of rotary evaporator. Then excess amount of solvents in the extracts were removed by rotary evaporator, with reduced pressure which was done by using a vacuum pump. The temperature of the rotary evaporator was set 50ºC. It run for 1 hours 10 minutes and the RPM was set 80 for evaporation process. After evaporation extract was transferred in a beaker. Rest of the extract was removed from the round bottle flask by using dichloromethane. Then extract was kept in hot air oven to get more dried extract. All beakers were covered with aluminum foil. The extract was then collected and stored in a cool (4˚C) dry place for further assay.

3.7 Principle of a Rotary Evaporator

A rotary evaporator is a device used in chemical laboratories for the efficient and gentle removal of solvents from samples by evaporation. When referenced in the chemistry research literature, description of the use of this technique and equipment may include the phrase "rotary evaporator", though use is often rather signaled by other language (e.g., "the sample was evaporated under reduced pressure"). Rotary evaporators are also used in molecular cooking for the preparation of distillates and extracts.

A simple rotary evaporator system was invented by Lyman C. Craig. It was first commercialized by the Swiss company Büchi in 1957. Other common evaporator brands are Heidolph, LabTech, Stuart, Hydrion Scientific, SENCO, IKA and EYELA. In research the most common form is the 1L bench-top unit, whereas large scale (e.g., 20L-50L) versions are used in pilot plants in commercial chemical operations.

Fig 3.1: Rotary Evaporator

3.8 Experimental Animals

Female Swiss albino mice of 3-4 weeks of age, weighing between 20–25 gram were used in pharmacological tests and females of the same strain in the LD_{50} calculation. The mice are kept in animal house at a standard environmental condition (temperature-22 ± 1°C relative humidity-55 ± 5% and 12h light/12 hr dark cycle).The animals were fed ad libitum with standard food and water except when fasting was required in the course of the study. The animals were acquired from the animal experimental centre of Jahangirnagar University, Depertment of Pharmacy, and Dhaka. Animals were kept in standard environmental conditions and had free access to feed and water.

3.9 Method of Identification of Animals

Each group consists of six animals. It was difficult to observe the biological response of six mice at a time receiving same treatment. It is quite necessary to identify individual animal of groups during treatment. The animals were individualized in the following way i.e. marked as

M1=mice 1, M2=mice 2, M3=mice3, M4=mice 4, M5=mice 5 & M6=mice 6.

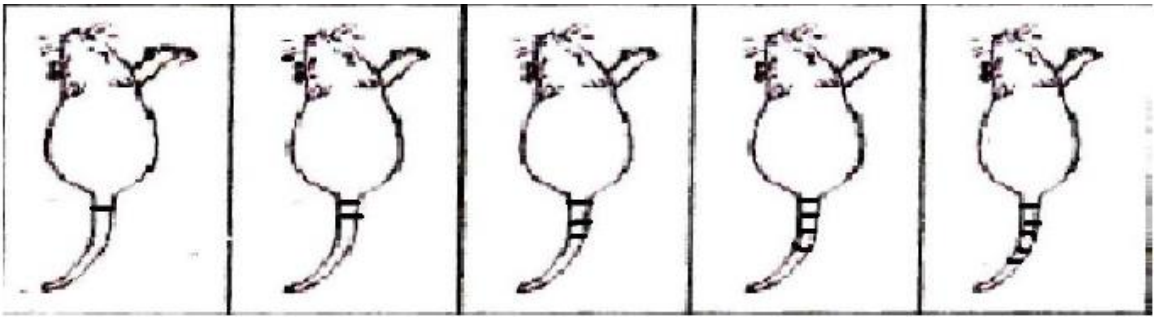


Fig 3.2: Identification of mice by mark

3.10 Pharmacological Evaluations

The activity of methanolic extract from *Thysanolaena maxima* on the central nervous system was then studied, using a battery of behavioral tests used in psychopharmacology. We analyzed the effect of different doses of the methanolic extracts (200mg/kg and 400 mg/kg, p.o.) from *Thysanolaena maxima* for their sedative and hypnotic activities. For testing sedative effect, the effect of extract on mice was qualified in one of the following tests.

Fig 3.3: Administration of dose into mice

3.11 Anxiolytic Activity Test

3.11.1 Materials for anxiolytic activity test:

- Analytical balance,
- Feeding needle: 1 c.c.

- Insulin syringes 100 units both disposable and nondisposable
- Hole board
- Lamp light
- Stop watch

3.11.2 Chemical agents used in anxiolytic activity test:

- 1% CMC or Carboxy Methyl Cellulose (Vehicle) 1g/100ml as negative control,

3.11.3 Standard drugs used in anxiolytic activity test:

- Diazepam 1mg/kg used as positive control in hole board test

3.11.4 Doses used in anxiolytic activity test of the extract:

- Methanolic extracts of *Thysanolaena maxima* at a dose of 200mg/kg and 400mg/kg of the crude extract are administered orally. Distilled water was used as a vehicle with plant methanolic extract for preparing different doses.

3.12 Hole-Board Test

Mice were individually placed in the centre of a perforated board and the number of head dips was registered during a 5 min. The perforated board test was made by using a wood floor board, 40 cm × 40 cm × 25 cm in which evenly spaced holes were made. The hole board represents a combination of a hole board originally designed to investigate explorative motivation in rodents and later on modified to evaluate cognitive functions The hole board itself consisted of a total of 16 holes, each 3 cm in diameter, were presented to the mouse in a flat space of 25 square centimeters. The animals were divided into negative control and test groups containing six mice in each group. Negative control group received vehicle (1% CMC solution) at a dose of 8 mg/kg body weight orally. The test groups received extracts *Thysanolaena maxima)* at the doses of 200 and 400mg/kg body weight orally. Each of the animals was transferred carefully to one corner of the field and the number of ambulation (expressed as the number of holes passed), head dipping and number of head poking was recorded for a period of 5 minutes at and posts 30 minutes intervals and was compared with the control animals.

Fig 3.4: Hole Board method

The number of explored holes provides a measure of the number of head dips.

1. Doses (in case of extract 200mg/kg & 400 mg/kg and in case of standerd 1mg/kg) were administered to mice.
2. Mice Placed on the board.
3. Monitor the number of dips and poke for five minutes.
4. Registered the number of dips and pokes.
5. Analysis the result.

Chapter 4
Result & Discussions

4.1 Results obtained by anxiolytic activity test of methanolic extract of *Thysanolaena maxima* by hole board test

The test is carried out to determine whether the extract of *Thysanolaena maxima* has any cognitive activity or not. The experimental findings that are noted are below-

Negative control group (1% CMC, 1g/100ml)

This group of animals only received vehicle (1% CMC) 1g/100ml orally. The observed total number of head poking is with a mean value of 8.5±2.39 (Mean ± SEM) during 5 minutes observation after 0 min of administration and 13.75±3.37 (Mean ± SEM) after 30 min of administration. The observed total number of head dipping with means value of 18.5±1.84 (Mean ± SEM) after 0 min of administration and 34.5±4.13 (Mean ± SEM) after 30 min of administration.

Positive control group (Diazepam, 1mg/kg)

This group of mice receives the standard drug Diazepam of 1mg/kg orally. The observed total number of head poking is with a mean value of 9.16±1.32 (Mean ± SEM) after 0 min of administration and 12.3±0.60 (Mean ± SEM) after 30 min of administration. The observed total number of head dipping with means value of 6.7±1.45 after 0 min of administration and 21.5±0.57 after 30 min of administration.

Test group-1 (Plant extract, 200mg/kg)

This test group of mice receives the plant extract of 200 mg/kg orally. The observed total number of head poking is with a mean value of 18±3.51 (Mean ± SEM) after 0 min of administration and 40.25±2.86 (Mean ± SEM) after 30 min of administration. The observed total number head dipping with mean value of 11.25±1.31 (Mean ± SEM) after 0 min of administration and 37.6±1.84 during 5 minutes observation after 30 min of administration.

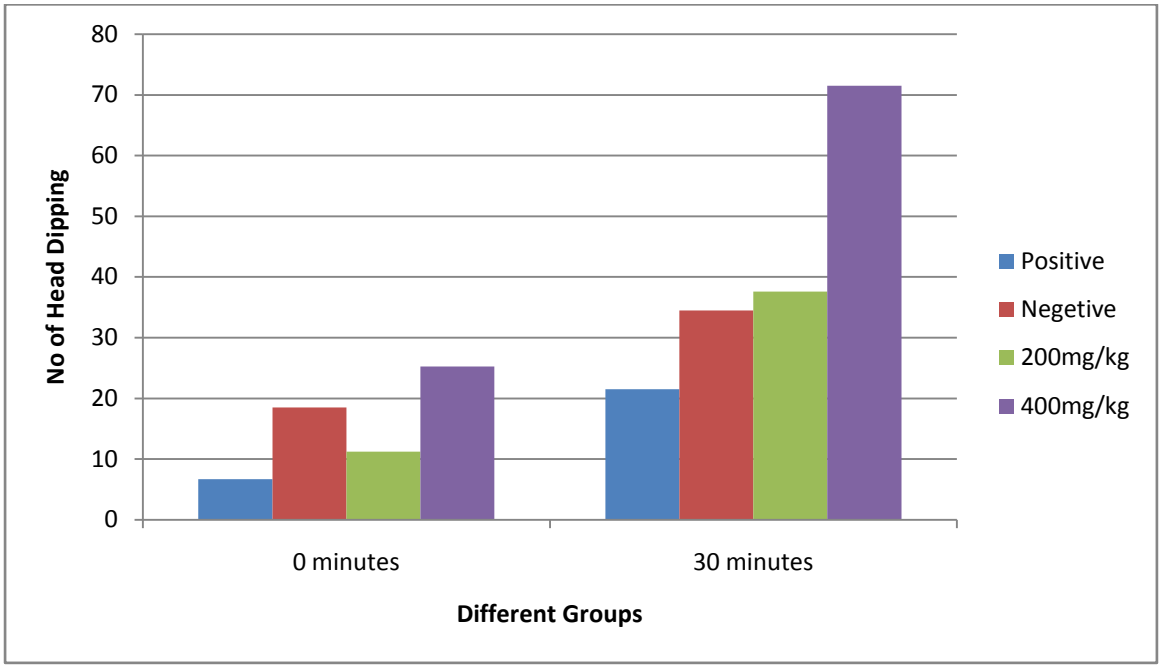
Test group-2 (Plant extract, 400mg/kg)

This group of mice receives the plant extract of 400 mg/kg orally. The observed total number of head poking is with a mean value of 6±1.47 (Mean ± SEM) after 0 min of administration and 17±4.45 (Mean ± SEM) after 30 min of administration. The observed total number of and head dipping with mean value of 25.25±3.01 (Mean ± SEM) after 0 min of administration and 71.5±8.08 (Mean ± SEM) after 30 min of administration.

4.2 Resuits obtained by observation of total No of Head Dipping

Table 4.1: Anxiolytic activity of plant extract of *Thysanolaena maxima* by hole board test in mice (No of Head Dipping)

Groups	Treatment	Dose	No of Head Dipping in 0 min	No. of Head Dipping in 30 min
Negative control	1% CMC	1g/100ml	18.5±1.84	34.5±4.13
Positive control	Diazepam	1 mg/kg	6.7±1.45	21.5±0.57
Group-1	Crude extract of *Thysanolaena maxima*	200 mg/kg	11.25±1.31	37.6±1.84
Group-2	Crude extract of *Thysanolaena maxima*	400 mg/kg	25.25±3.01	71.5±8.08

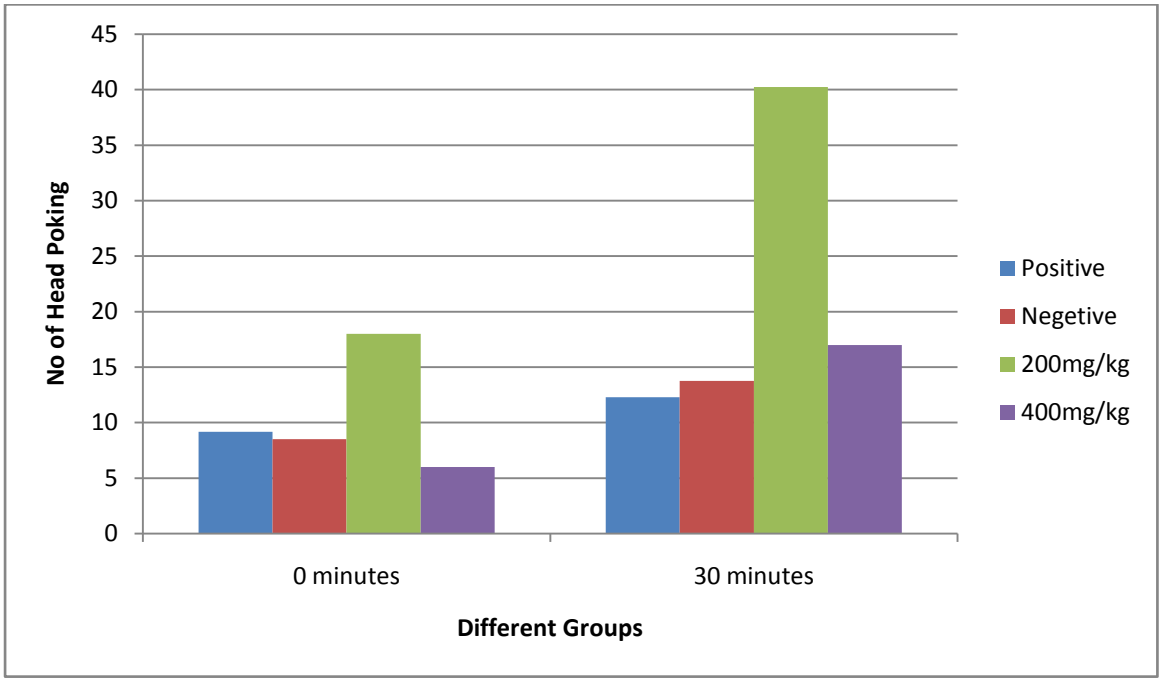


Figure–4.1: Graphical presentation of anxiolytic activity of plant extract of *Thysanolaena maxima* by observation of no of Head Dipping

4.3 Resuits obtained by observation of total No of Head Poking

Table–12: Anxiolytic activity of plant extract of *Thysanolaena maxima* by hole board test in mice (No of Head Poking)

Groups	Treatment	Dose	No of Head Poking in 0 min	No. of Head Poking in 30 min
Negative control	1% CMC	1g/100ml	8.5±2.39	13.75±3.37
Positive control	Diazepam	1 mg/kg	9.16±1.32	12.3±0.60
Group-1	Crude extract of *Thysanolaena maxima*	200 mg/kg	18±3.51	40.25±2.86
Group-2	Crude extract of *Thysanolaena maxima*	400 mg/kg	6±1.47	17±4.45



Figure–4.1: Graphical presentation of anxiolytic activity of plant extract of *Thysanolaena maxima* by observation of No of Head Poking

4.4 Discussion:

For the production of commercial drugs or in the improvement of lead compounds, it has been built that medicinal plants are good reservoir of it. Most of the drugs that are used for depression affect the quality life of sick person. Oppositely, herbal medicines have less toxicity, good absorption and have a lower side effect profile. So, this has been used since very old times. Hence it is necessary to create efforts to represent the new medicinal plants for production of cheaper and less toxic drugs. That's why a number of experiments have been done on the plant extract of *Thysanolaena maxima* to determine its anxiolytic activity on mice. From the results, it was seen that the plant extract contains CNS depressant or anxiolytic activity. The standard (diazepam) was used in this study as standard compared with the control and extract. From our best knowledge, this is the first report of CNS depressant and anxiolytic activity of *Thysanolaena maxima*. There is increase of frequency of head dipping and head poking after administration of 200 mg/kg extract with time (at 0 minutes and after 30 minutes). This Frequency of head dipping and (200 mg/kg) is comparable to that of the standard drug diazepam at a dose of 1 mg/kg (ip) and control (1% CMC). The hole-board model indicates that the head-dipping and poking behaviour is sensitive to the emotional state of animals and suggests that the expression of

the anxiolytic state in animals may be reflected by an increase in head-dipping behavior. So *Thysanolaena maxima* showed anxiolytic effect in 200 mg/kg. The frequency of head dipping is far more increased in 400 mg/kg compared both control and standard with time (at 0 minutes and after 0 minutes). So *Thysanolaena maxima* showed potant anxiolytic effect in both 200 mg/kg and 400 mg/kg.

Chapter 5

Conclusion

Conclusion

Approximately two-thirds of the anxious patients respond to the currently available treatments but the magnitude of improvement is still disappointing, besides, they also produce various systemic side effects and exhibit dependence and tolerance on chronic treatment which now have become a major concern about the use of currently used medicines. Our results revealed that the methanolic extract of *Thysanolaena maxima* appears to contain substance(s) that possess potent anxiolytic activity. *Thysanolaena maxima* has medicinal value on psychological aspect that shown potent depressive and anxiolytic effect. The crude extract dose showed significant result when it is evaluated by hole board method. The reduction is significant when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, diazepam 1mg/kg. Now it can be concluded on the basis of results obtained from investigation that the plant may be useful as CNS depressant agent. But our work was only preliminary effort. It will require additional detailed advanced investigation. Future studies will be focused on the neurobiological mechanisms of action and a possible interaction of Thysanolaena maxima with the phytoconstituent(s) responsible for the observed central effects has to be isolated and identified.

Chapter 6
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