

**Study of Pharmacological Activities of Methanolic Extract of
*Phyla Nodiflora***

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This Thesis Paper is submitted to the Department of Pharmacy, East West University, Bangladesh, in partial fulfillment of the requirements for the Degree of Masters of Pharmacy.

CERTIFICATE

This is to certify that, the research work on ‘Study of Pharmacological Activities of Methanolic Extract of *Phyla Nodiflora*’ submitted to Department of Pharmacy, East West University, Jahurul Islam city, Aftabnagar, Dhaka-1212, in partial fulfilment of the requirements for the degree of Masters of Pharmacy (M. Pharm) was carried out by Md. Firoz Hossen (ID: 2013-3-79-021) under the guidance and supervision and that not part of thesis has been submitted for any other degree. We further certify that all the sources of information of this connection are duly acknowledged.

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ABSTRACT

Purpose: The research work was carried out to determine the pharmacological activities of methanolic extract of *Phyla Nodiflora*.

Method: Methanolic extract was administered orally to the animal model (*Swiss albino*) and the effects were determined by comparing with respect to control group which were treated with 5% CMC. For every experiment positive control was used. Different experiments were used to determine the pharmacological profile which was collected from internationally published publications and journals.

Result: The Laxative Effect was evaluated by Charcoal meal GI transit test where methanolic extract at dose of 800 mg/kg body weight increase the charcoal transit which compare with positive control.

The CNS activity was evaluated by open field method and hole board test. In the open field method and hole board experiment the crude extract of *Phyla Nodiflora*. (200mg/kg, 400mg/kg & 800mg/kg) dose dependently reduces the number of peripheral locomotion, central locomotion and leaning in the open field test and reduces the number of head dipping and head poking in the hole board test. The reduction is significant (***) ($p < 0.001$) when it is compared to the standard drug.

The aim of the study was also to investigate the possible toxicity of the plant *Phyla Nodiflora* and especially to establish the safety of the methanolic extract of this plant by focusing on its acute and chronic toxicity in mice.

Conclusion: After summarize all the results it can say that *Phyla Nodiflora* may have several pharmacological activities but to prove the hypothesis it need further higher studies.

Keywords: *Phyla Nodiflora*, Laxative effect, Neuropharmacological effect and Toxicity test.

Dedication

This research work is dedicated to my beloved parents,
honorable faculties and loving friend.

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Annexure

List of Abbreviation	Full Meaning
AGA	American Gastroenterological Association
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ALP	Alkaline Phosphatase
ANOVA	One-way Analysis of Variance
CAM	Complementary & Alternative Medicine
CBC	Complete Blood Count
CMC	Carboxy Methyl Cellulose
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
EVF	Erythrocyte Volume Fraction
GPT	Glutamate Pyruvate Transaminase
HCT	Hematocrit
ICDDR, B	International Centre for Diarrhoeal Disease and Research, Bangladesh
LFTs or LFs	Liver Function Tests
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Cell Volume
MS	Multiple sclerosis
NCCAM	National Center for Complementary & Alternative Medicine
PAG	Periaqueductal Grey Matter
PCV	Packed Cell Volume
PNS	Peripheral Nervous System
PT	Prothrombin Time
PV	Polycythemia Vera

RBC	Red Blood Cell
RDW or RCDW	Red Blood Cell Distribution Width
RPM	Rotation Per Minute
SALP	Serum Alkaline Phosphatase
SEM	Standard Error Mean
SGOT	Serum Glutamate Oxaloacetate Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
SPSS	Statistical Package for the Social Science
WBC	White Blood Cell

Chapter 1

INTRODUCTION

1.Introduction

1.1. Medicinal Plant

The medicinal use of plants is probably as old as mankind itself. Plants have continued to be a valuable source of natural products for maintaining human health, as studies on natural therapies have intensified. More than 150,000 plant species have been studied, and several of them contain therapeutic substances. The use of plant compounds for pharmaceutical purposes has gradually increased. According to the World Health Organization medicinal plants are probably the best source of a variety of drugs. About 80 % of individuals in developed countries use traditional medicine containing compounds derived from medicinal plants (Varalakshmi, et.al. 2011). Medicinal plants, defined as plants used for maintaining health and/or treating specific ailments, are used in a plethora of ways in both allopathic and traditional systems of medicine in countries across the world. Even people using only allopathic medicine throughout their lives are likely to be somewhat medicinal plant reliant as 20-25% of drugs prescribed are plant derived (Hall, et.al. 2012).

1.1.1. Definitions of medicinal plants

A considerable number of definitions have been proposed for medicinal plants. According to the WHO, “A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis.” When a plant is designated as ‘medicinal’, it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. “Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes” (Ghani, 2003).

Herbal medicines have been utilized for many purposes, particularly in medical care as antiasthmatics(86.79 %),anti-rheumatics (62 %), diuretics (60.22 %),antiinflammation (29.62 %),anticancer(9.75 %),antidiabetics(8.33 %), antimicrobials, antifungals, antioxidants, antiallergy, analgesics, anti-obesity and antihypertention. In dental care it has been employed as anticariogenic, analgesic, local anesthetic, wound healing agents, anti-inflammation and recurrent aphthous stomatitis treatment etc.

1.1.2 Importance of Medicinal Plant

Plants are the tremendous source for the discovery of new products with medicinal importance in drug development. Today several distinct chemicals derived from plants are important drugs, which are currently used in one or more countries in the world. Herbal medicines have been utilized for many purposes, particularly in medical care as antiasthmatics (86.79 %), anti-rheumatics (62 %), diuretics (60.22 %), antiinflammation (29.62 %), anticancer (9.75 %), antidiabetics (8.33 %), antimicrobials, antifungals, antioxidants, antiallergy, analgesics, anti-obesity and antihypertention. In dental care it has been employed as anticariogenic, analgesic, local anesthetic, wound healing agents, anti-inflammation and recurrent aphthous stomatitis treatment etc.

The primary metabolites, in contrast, such as phytosterols, acyl lipids, nucleotides, amino acids, and organic acids, are found in all plants and perform metabolic roles that are essential and usually evident. Although noted for the complexity of their chemical structures and biosynthetic pathways, natural products have been widely perceived as biologically insignificant and have historically received little attention from most plant biologists.

Plants produce a vast and diverse assortment of organic compounds, the great majority of which do not appear to participate directly in growth and development. These substances, traditionally referred to as secondary metabolites, often are differentially distributed among limited taxonomic groups within the plant kingdom. The secondary metabolites are known to play a major role in the adaptation of plants to their environment and also represent an important source of pharmaceuticals. Their functions, many of which remain unknown, are being elucidated with increasing frequency. Secondary metabolites are economically important as drugs, flavor and fragrances, dye and pigments, pesticides, and food additives. Many of the drugs sold today are simple synthetic modifications or copies of the naturally obtained substances.

Based on their biosynthetic origins, plant natural products can be divided into three major groups: the terpenoids, the alkaloids, and the phenolic compounds. All terpenoids, including both primary metabolites and more than 25,000 secondary compounds, are derived from the five-carbon precursor isopentenyl diphosphate (IPP). The 12,000 or so known alkaloids, which contain one or more nitrogen atoms, are biosynthesized principally from amino acids. The 8000 or so phenolic compounds are formed by way of either the shikimic acid pathway or the malonate/acetate pathway (Ghani, 2003).

1.1.3 Medicinal plants & Traditional Medicine Practice in Bangladesh

The plants which are useful for healing several diseases are called medicinal plant. There are 722 medicinal plants in our country. Bangladesh possesses a rich flora of medicinal plants. Out of the estimated 5000 species of different plants growing in this country more than a thousand are regarded as having medicinal properties. Out of them, more than a thousand have been claimed to possess medicinal and poisonous properties, of which 546 have recently been enumerated with their medicinal properties and therapeutic uses. In addition to possessing various other medicinal properties, 257 of these medicinal plants have been identified as efficacious remedies for diarrhoeal diseases and 47 for diabetes (Ghani, 2003).

Use of these plants for therapeutic purposes has been in practice in this country since time immemorial. Continuous use of these plants as items of traditional medicine in the treatment and management of various health problems generation after generation has made traditional medicine an integral part of the culture of the people of this country. As a result, even at this age of highly advanced allopathic medicine, a large majority (75-80%) of the population of this country still prefer using traditional medicine in the treatment of most of their diseases even though modern medical facilities may be available in the neighbourhood.

Traditional medical practice among the tribal people is mainly based on the use of plant and animal parts and their various products as items of medicine. The medicaments, prepared from plant materials and other natural products sometimes also include some objectionable substances of animal origin. They are dispensed in a number of dosage forms like infusions, decoctions, pastes, moulded lumps, powders, dried pills, creams and poultices. Diets are strictly regulated (Hussain, *et.al.*, 2012).

1.1.4 Toxicity aspects of use of herbal preparations

Currently, there is an ongoing world-wide “green” revolution which is mainly premised on the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs (Williamson et al., 1996). Many writers claim that it is assumed that “all things natural are good” (Gaillard and Pepin, 1999) and, generally, the extensive traditional use of herbal products is not assumed to be based on a comprehensive well documented logic, but rather on empirical wisdom accumulated over many years, often arrived at through trial and error and transmitted orally from generation to generation. This traditional methodology has enabled those herbal medicines

producing acute and obvious signs of toxicity to be well recognized and their use avoided. However, the premise that “traditional use of a plant for perhaps many hundreds of years establishes its safety does not necessarily hold true”. The more subtle and chronic forms of toxicity, such as carcinogenicity, mutagenicity, and hepatotoxicity, may well have been overlooked by previous generations and it is these types of toxicity that are of most concern when assessing the safety of herbal remedies (Williamson *et al.*, 1996).

1.1.5. Causes of toxicity with herbal products

All chemicals may be considered toxic under certain conditions, e.g. even pure water when inhaled is rapidly absorbed across the lung alveoli to cause lysis of red blood cells. But some chemicals present a greater hazard than others (Pascoe, 1983). A large number of plants contain appreciable levels of biosynthetically produced chemical substances and many of these have either been reported to be toxic to humans or are predictably toxic based on extensive animal or *in vitro* studies (Tomlinson and Akerele, 1998).

Toxicity with medicinal plant products may arise in various ways, but in general two categories of causes can be distinguished:

- In the first category, as previously mentioned, the toxicity may be as a result of exposure to intrinsic ingredients of some medicinal plants. Examples of some more important classes of ingredients implicated here include: *pyrrolizidinealkaloids*, which are said to be hepatocarcinogens; *aristolochic acid I*, said to be mutagenic and carcinogenic; *phorbol esters*, which are tumor promoters and vesicant to the skin; *carboxyatractyloside*, a deadly toxic compound; *amygdalin*, a cyanogenic compound with many undesired effects; etc. (Gaillard and Pepin, 1999; Tomlinson and Akerele, 1998). In addition, several studies conducted on flavonoids indicate that, besides their apparently beneficial health effects, they may also induce mutagenicity and genotoxicity (e.g. quercetin) in both bacterial and mammalian experimental systems (Skibola and Smith, 2000).
- The second category of causes of toxicity of herbal medicines is more extrinsic or non-associated with the plant active constituents. In this category, the toxicity is a result of exposure

to plant products contaminated with excessive or banned pesticides, microbial contaminants, heavy metals or chemical toxins, or with substituted ingredients. The pesticide, heavy metal and microbial contaminants may be linked to the source, collection or processing of the herbal materials (e.g.in contaminated environments).

1.1.6. Prevalence of toxicity with herbal products

Different retrospective studies done over the last 20 years indicated that the incidence of deaths occurring due to exposure to plants (as a proportion of total patients poisoned by traditional plant medicine) was about 1.5% in France, 5% in Belgium, 6.5% in Italy, 7.2% in Switzerland and 6% in Turkey (Gaillard and Pepin, 1999). The total number of deaths due to exposure to plants throughout the world however, is very difficult to establish and must certainly be underestimated since all cases of such deaths were, from analytical and forensic points of view, not always well documented and thus, rarely published. Nevertheless, it seems that death due to plant poisoning might be more important than other causes of poisonings. For instance, in South Africa, 2% of the people admitted for acute poisoning died compared to 15% of the patients poisoned with traditional plant medicine (Gaillard and Pepin, 1999).

From published reports, it appears that side effects or toxic reactions, of any form but associated with herbal medicines, are rare (Tomlinson and Akerele, 1998). This may be because herbal medicines are generally safe, that adverse reactions following their use are underreported, or because the side effects are of such a nature that they are not reported (Tomlinson and Akerele, 1998; Gaillard and Pepin, 1999).

1.2. Constipation

Constipation is a condition in which a person has fewer than three bowel movements a week or has bowel movements with stools that are hard, dry, and small, making them painful or difficult to pass. People may feel bloated or have pain in their abdomen. Some people think they are constipated if they do not have a bowel movement every day. Most people get constipated at some point in their lives. Constipation can be acute, which means sudden and lasting a short time, or chronic, which means lasting a long time, even years. Most constipation is acute and not

dangerous. Understanding the causes, prevention, and treatment of constipation can help many people take steps to find relief.

Constipation is one of the most common gastrointestinal problems. People of any age, race, or gender can get constipated. Constipation most commonly occur in women, adults ages 65 and older, and people in lower socioeconomic classes. Constipation is also a common problem during pregnancy, following childbirth or surgery, or after taking medications to relieve pain from things such as a broken bone, tooth extraction, or back pain (Higgins et al., 2004). Constipation is also chronic and it may cause piles, fissure, anal abscess/fistula etc.

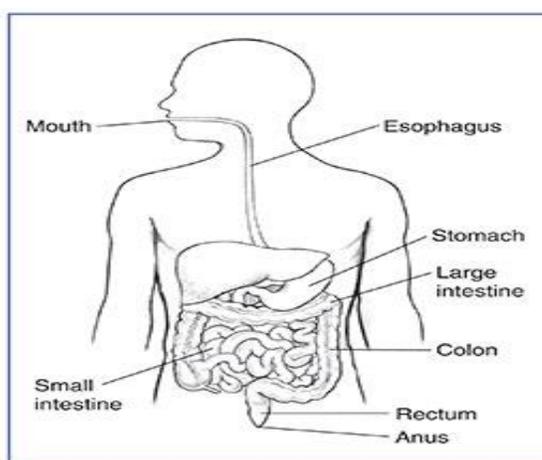


Figure 1.1: Human Gastrointestinal Tract

1.2.1 Symptoms of constipation can include:

- Infrequent bowel movements and/or difficulty having bowel movements
- Swollen abdomen or abdominal pain
- Pain
- Vomiting

1.2.2. Causes of Constipation

Constipation is caused by stool spending too much time in the colon. The colon absorbs too much water from the stool, making it hard and dry. Hard, dry stool is more difficult for the muscles of the rectum to push out of the body.

Common factors or disorders that lead to constipation are

- Inadequate water intake
- Inadequate fiber in the diet
- A disruption of regular diet or routine; traveling
- Inadequate activity or exercise or immobility
- Eating large amounts of dairy products
- Stress
- Resisting the urge to have a bowel movement, which is sometimes the result of pain from hemorrhoids
- Hypothyroidism
- Neurological conditions such as Parkinson's disease or multiple sclerosis
- Depression
- Eating disorders
- Irritable bowel syndrome
- Pregnancy
- Colon cancer

1.2.3. Medications that can cause constipation include

- ✓ Pain medications (analgesic), especially narcotics. e.g.: Morphine, Methadone.
- ✓ Antacids that contain aluminum and calcium. e.g.: Calcium and Magnesium Hydroxide.
- ✓ Calcium channel blockers, which are used to treat high blood pressure and heart disease. e.g.: Nifedipine, Nicardipine etc.
- ✓ Drugs that treat Parkinson's disease—a disorder that affects nerve cells in a part of the brain that controls muscle movement—because these medications also affect the nerves in the colon wall. e.g.: Levodopa.
- ✓ Antispasmodics—medications that prevent sudden muscle contractions.e.g.: Alverine, Mebeverine, Drotaverine etc.
- ✓ Some antidepressants. e.g.: Amitriptyline, Amoxapine, Desipramine etc.
- ✓ Iron supplements. e.g.: Iron tablets.

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- ✓ Diuretics—medications that help the kidneys remove fluid from the blood. e.g.: Frusemide, Spironolactone, Indapamide etc.

Constipation can also be caused by overuse of over-the-counter laxatives. A laxative is medication that loosens stool and increases bowel movements. Although people may feel relief when they use laxatives, they usually must increase the dose over time because the body grows reliant on laxatives to have a bowel movement. Overuse of laxatives can decrease the colon's natural ability to contract and make constipation worse. Continued overuse of laxatives can damage nerves, muscles, and tissues in the large intestine.

1.2.4. Neurological and Metabolic Disorders

Certain neurological and metabolic disorders can cause food to pass through the digestive system too slowly, leading to constipation. Neurological disorders, such as spinal cord injury and Parkinsonism, affect the brain and spine. Parkinsonism is any condition that leads to the types of movement changes seen in Parkinson's disease. Metabolic disorders, such as diabetes and hypothyroidism, disrupt the process the body uses to get energy from food. Hypothyroidism is a disorder that causes the body to produce too little thyroid hormone, which can cause many of the body's functions to slow down.

1.2.5. GI Tract Problems

Some problems in the GI tract can compress or narrow the colon and rectum, causing constipation.

These problems include

- Adhesions—bands of tissue that can connect the loops of the intestines to each other, which may block food or stool from moving through the GI tract.
- Diverticulosis—a condition that occurs when small pouches, or sacs, form and push outward through weak spots in the colon wall; the pouches are called diverticula.

- Polyps- Polyps are benign growths that can grow, bleed, and become cancerous. Colorectal cancer is the third most common cancer in the U.S. It often causes bleeding that is not noticeable with the naked eye.
- Anal fissure- A small cut or tear in the tissue lining the anus similar to the cracks that occur in chapped lips or a paper cut. Fissures are often caused by passing a large, hard stool and can be painful.

1.2.6. Functional GI Disorders

Functional GI disorders are problems caused by changes in the GI tract works. Functional GI disorders often results from problems with muscle activity in the colon or anus that delay stool movement. Functional GI disorders are diagnosed in people who have had symptoms for at least 6 months and meet the following criteria for the last 3 months before diagnosis. Irritable bowel syndrome (IBS) is a functional GI disorder with symptoms that include abdominal pain or discomfort, often reported as cramping, along with diarrhea, constipation, or both (Longstreth et al., 2006).

1.2.7. Constipation Complications

For those people who suffer from chronic or severe constipation, complications can occur. The complications of constipation range from nagging to serious. A visit to the doctor for a diagnosis and treatment is the best way to avoid the complications of constipation.

1.2.8. Rectal Complications of Constipation

Hemorrhoids are a common complication of constipation. Hemorrhoids are inflamed and swollen vascular tissues that form close to the anus. The hemorrhoids can cause itching, pain or burning and are very uncomfortable. Hemorrhoids can also cause localized bleeding at the rectum or anus because of the vascular nature of the tissue. Hemorrhoids worsen when the hardened stools create pressure while attempting to have a bowel movement.

1.2.9. The Complication of Fecal Impaction

A fecal impaction is the accumulated and hardened stool in the large intestine as a result of constipation. The symptoms of a fecal impaction include no solid stool being passed and liquid stool leaking from the colon. Only liquid can pass, because the colon is impacted and blocked. A

fecal impaction can be a serious complication, especially if it causes an intestinal blockage and remains untreated.

Complications from constipation can usually be minimized if constipation is managed. The best treatment for complications from constipation is to alleviate the constipation. For those who experience complications, a doctor can not only diagnose the problem, but will treat it as well. Constipation can be controlled with diet, fluids, activity and medications when necessary, but a trip to the doctor is recommended.

1.2.10. Diagnosis of Constipation

To diagnose the cause of constipation, the health care provider will take a medical history and order specific tests. The tests ordered depend on how long the person has been constipated; how severe the constipation is; the person's age; and whether the person has had blood in stools, recent changes in bowel habits, or weight loss. Most people with constipation do not need extensive testing and can be treated with changes in diet and exercise.

1.2.11. Diagnostic Tests

Additional testing is usually reserved for older adults and people with severe symptoms, sudden changes in the number and consistency of bowel movements, or blood in the stool. Additional tests that may be used to evaluate constipation include

- Blood test
- Lower GI series
- Colorectal transit studies
- Anorectal function tests
- Defecography (It is a radiological test that allows the doctor to visualize what occurs in empty rectum. This test may be awkward but provides valuable information that will aid in diagnosing GIT problem).
- Flexible sigmoidoscopy or colonoscopy (It is a test that uses a flexible, narrow tube with a light and tiny camera on one end, called a sigmoidoscope or scope, to look inside the rectum and the lower, or sigmoid, colon. Flexible sigmoidoscopy can show irritated or swollen

tissue, ulcers, and polyps—extra pieces of tissue that grow on the inner lining of the intestine).



Figure 1.2: Flexible sigmoidoscopy

For the test, the person will lie on a table while the health care provider inserts a sigmoidoscope into the anus and slowly guides it through the rectum and into the sigmoid colon.

1.2.12. Treatment of Constipation

Treatment for constipation depends on the cause, severity, and duration of the constipation and may include one or more of the following:

- changes in eating, diet, and nutrition
- exercise and lifestyle changes
- medication
- surgery
- biofeedback

First-line treatments for constipation include changes in eating, diet, and nutrition; exercise and lifestyle changes; and laxatives. People who do not respond to these first-line treatments should talk with their health care provider about other treatments.

1.2.13. Medication

Classification

I. Luminally active agents

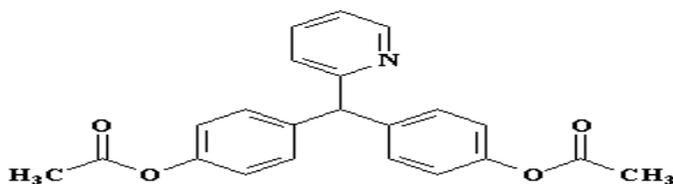
- i) Bulk forming - Dietary fibre, psyllium, ispaghula, methyl cellulose
- ii) Stool softener - Dioctyl sodiumsulphosuccinate (Docusates, Doss)
- iii) Lubricants - Liquid paraffin
- iv) Osmotic - Magnesium sulphate, Magnesium hydroxide, Sodium sulphate, Sodium potassiumtartrate, Lactulose, Sorbitol, Mannitol.

II. Stimulant (Contact) Purgatives

- i) Diphenylmethanes - Phenolphthalein, bisacodyl
- ii) Anthraquinones - Senna, cascara, rhubarb, aloes, danthron
- iii) Fixed oil - Castor oil

III. Prokinetic agents - 5 HT4 agonists e.g. Tegaserod

- Opioid receptor antagonists



Bisacodyl
C₂₂H₁₉NO₄

1.3. Nervous System

The human nervous system is perhaps the most complex system of any organism. The human brain alone contains over 100 billion nerve cells, and each nerve cell can have up to 10,000 connections to other nerve cells. This means that a nerve impulse—an electrochemical signal to or from the brain could travel along 10^{15} possible routes. The nervous system has two major divisions: the central nervous system (CNS) and the peripheral nervous system (PNS).

Early researchers made this distinction based on where nervous tissue was located in the body centrally or away from the center (peripherally). Together, the central nervous system and the peripheral nervous system control sensory input, integration, and motor output.

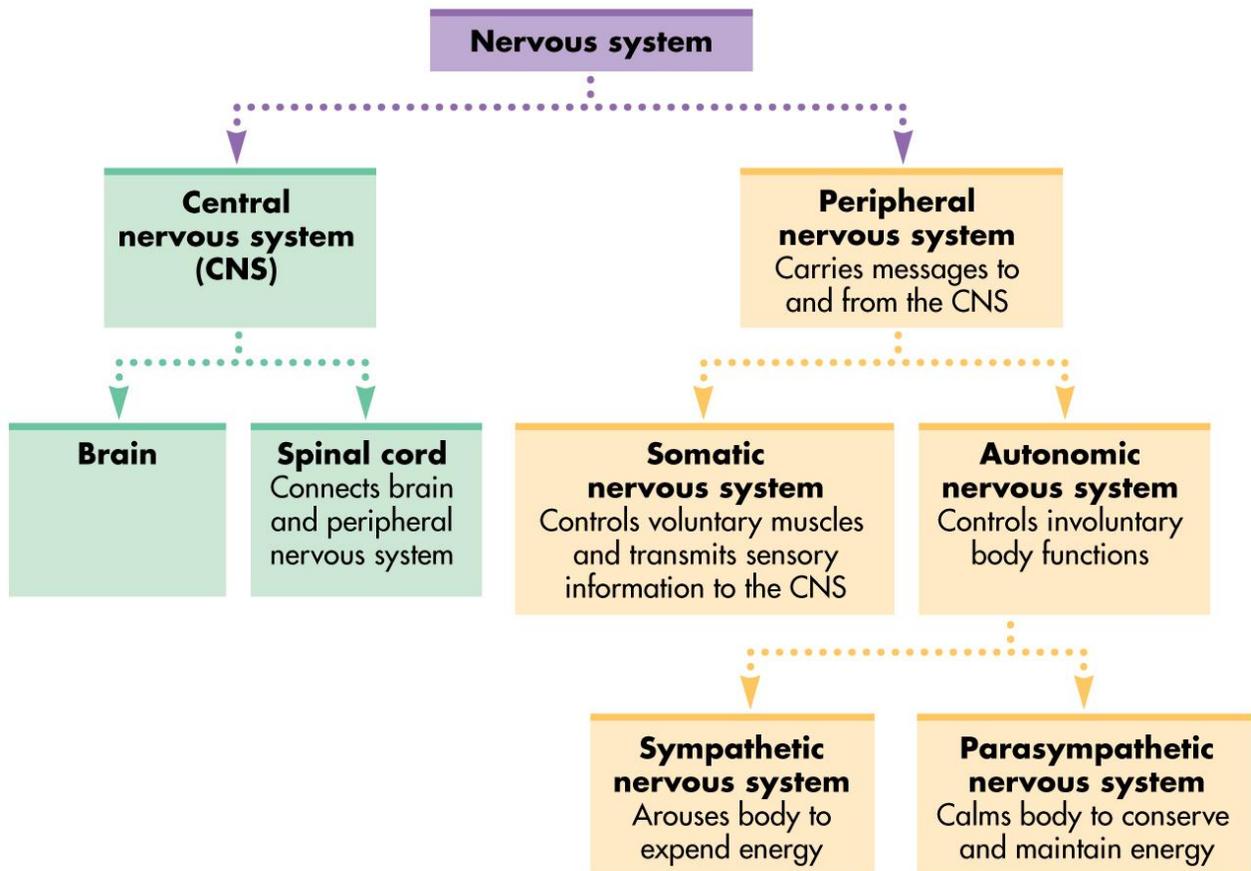


Figure1.3: Organization of the Human Nervous System.

1.3.1. The central Nervous System

The "Central Nervous System", comprised of brain, brainstem, and spinal cord. The central nervous system (CNS) represents the largest part of the nervous system, including the brain and the spinal cord. Together, with the peripheral nervous system (PNS), it has a fundamental role in the control of behavior. The CNS is conceived as a system devoted to information processing, where an appropriate motor output is computed as a response to a sensory input. CNS is protected by Bone (skull, vertebrae). They are also wrapped up in three protective membranes called meninges (spinal meningitis is infection of these membranes). Spaces between meninges filled with cerebrospinal fluid for cushioning and protection. This fluid also found within central canal of the spinal cord and ventricle of brain. (Kandel, et.al. 2000)

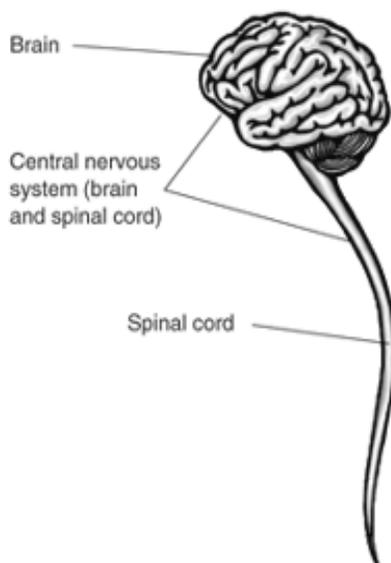


Figure1.4: Central Nervous System

Parts of Central Nervous System

- Brain
- Medulla
- Pons
- Cerebrum
- Cerebellum
- Spinal Cord

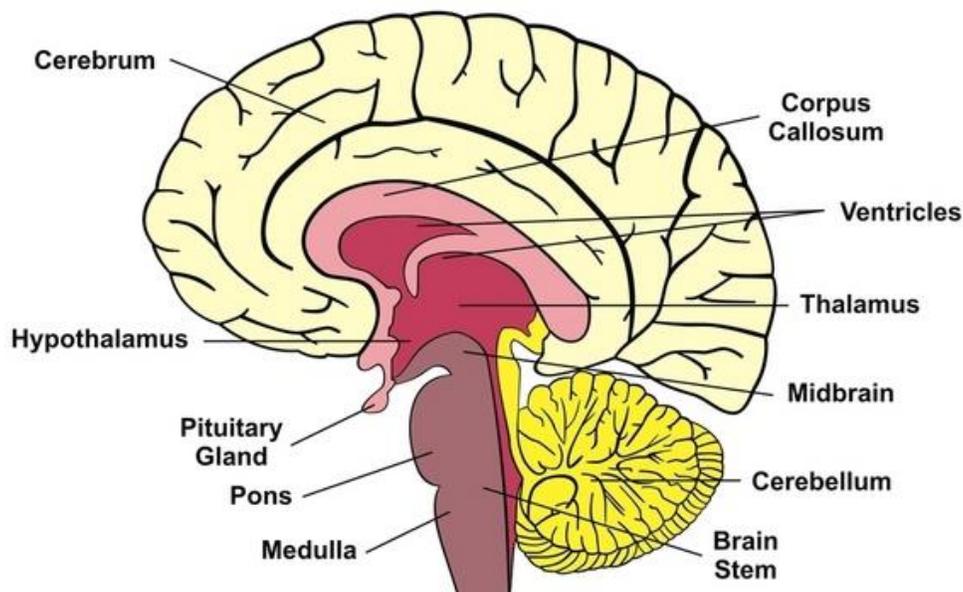


Figure1.5: Human Brain

1.3.2. Peripheral Nervous System:

The peripheral nervous system includes nerves that carry sensory messages to the central nervous system and nerves that send information from the CNS to the muscles and glands. The peripheral nervous system is further divided into the somatic system and the autonomic system. The peripheral nervous system includes 12 cranial nerves 31 pairs of spinal nerves.

Somatic nervous system and Autonomic nervous system are the part of peripheral nervous system

Somatic Nervous System: The somatic system consists of nerves that carry sensory information to the central nervous system, and nerves that carry instructions from the central nervous system to the skeletal muscles.

Autonomic Nervous System: The autonomic system controls glandular secretions and the functioning of the smooth and cardiac muscles. The sympathetic and parasympathetic divisions of the autonomic system often work in opposition to each other to regulate the involuntary processes of the body. Involuntary processes, such as heartbeat and peristalsis, are those that do not require or involve conscious control.

1.3.3. Nerve cells

Neurons or nerve cells carry out the functions of the nervous system by conducting nerve impulses. They are highly specialized. If a neuron is destroyed, it cannot be replaced because neurons do not go through mitosis. Each neuron has three basic parts like, cell body (soma), one or more dendrites, and a single axon.

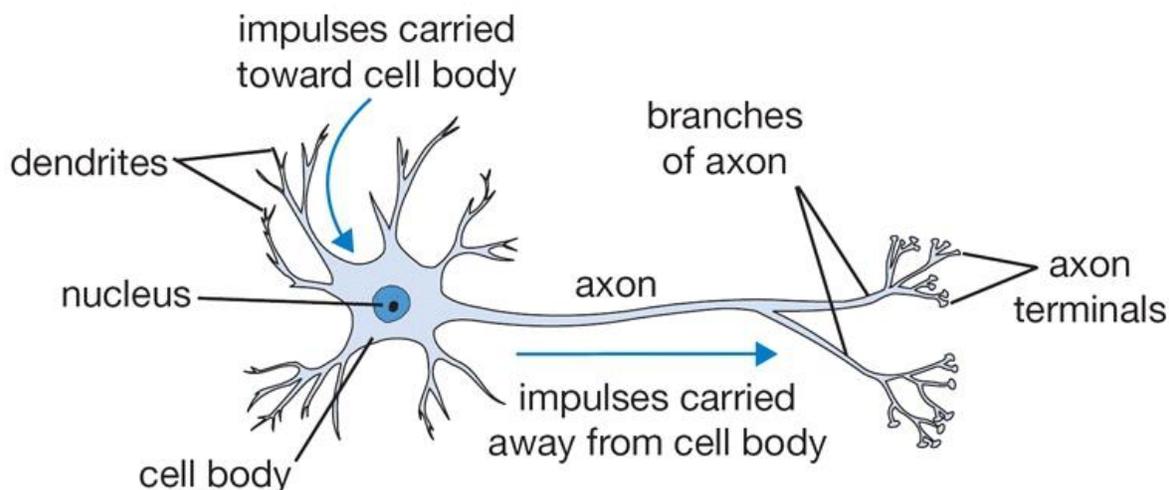


Figure1.6: Neuron

Cell Body or Soma:

In many ways, the cell body is similar to other types of cells. It has a nucleus with at least one nucleolus and contains many of the typical cytoplasmic organelles. It lacks centrioles. Because centrioles function in cell division, the fact that neurons lack these organelles is consistent with the amitotic nature of the cell. It is the metabolic center of the neuron. It gives rise to further two processes, dendrites and axon.

Axon:

Cell body gives rise to a tubular process which is the main conducting unit of the neuron, capable of conveying information at great distances by propagating transient electrical signal called action potential. Many axons are surrounded by a segmented, white, fatty substance called myelin or the myelin sheath. Myelinated fibers make up the white matter in the CNS, while cell bodies and unmyelinated fibers make the gray matter. The unmyelinated regions between the

myelin segments are called the nodes of ranvier. Thus, axons are of two types, myelinated and non-myelinated.

Dendrites:

Dendrites and axons are cytoplasmic extensions, or processes, that project from the cell body. They are sometimes referred to as fibers. Dendrites are usually short and branching, which increases their surface area to receive signals from other neurons. The number of dendrites on a neuron varies (Martini, et.al. 2003).

1.3.4. Synapse

The synapse is a small gap separating neurons. The synapse consists of a presynaptic ending that contains neurotransmitters, mitochondria and other cell organelles, a postsynaptic ending that contains receptor sites for neurotransmitters and a synaptic cleft or space between the presynaptic and postsynaptic endings. It is about 20nm wide.

1.3.5. Different Central Nervous System Disorders

- ✓ **Alzheimer's disease**-A progressive, degenerative disease that occurs in the brain and results in impaired memory, thinking, and behavior.
- ✓ **Bradykinesia**- Slowness of movement.
- ✓ **Bradyphrenia**-Slowness of thought processes
- ✓ **Cerebral embolism**- A brain attack that occurs when a wandering clots (embolus) or some other particle forms in a blood vessel away from the brain - usually in the heart.
- ✓ **Cerebral hemorrhage**- A type of stroke occurs when a defective artery in the brain bursts, flooding the surrounding tissue with blood.
- ✓ **Cerebral thrombosis**- The most common type of brain attack; occurs when a blood clot (thrombus) forms and blocks blood flow in an artery bringing blood to part of the brain.
- ✓ **Delusions**- A condition in which the patient has lost touch with reality and experiences hallucinations and misperceptions.
- ✓ **Dementia**– It is not a disease itself, but group of symptoms that characterize diseases and conditions; it is commonly defined as a decline in intellectual functioning that is severe enough to interfere with the ability to perform routine activities.
- ✓ **Epilepsy** (Also called seizure disorder)-A brain disorder involving recurrent seizures.

- ✓ **Euphoria**– A feeling of well-being or elation; may be drug-related.
- ✓ **Guillain-Barré syndrome**- A disorder in which the body's immune system attacks part of the nervous system.
- ✓ **Headache (primary)**-Includes tension (muscular contraction), vascular (migraine), and cluster headaches not caused by other underlying medical conditions.
- ✓ **Headache (secondary)**-Includes headaches that result from other medical conditions. These may also be referred to as traction headaches or inflammatory headaches.
- ✓ **Meningitis**-An inflammation of the meninges, the membranes that cover the brain
- ✓ **Multiple sclerosis (MS)**-A disease of the central nervous system that is an unpredictable condition that can be relatively benign, disabling, or devastating, leaving the patient unable to speak, walk, or write.
- ✓ **Parkinson's disease (PD)**-The most common form of parkinsonism; a slowly progressing, degenerative disease that is usually associated with the following symptoms, all of which result from the loss of dopamine-producing brain cells: tremor or trembling of the arms, jaw, legs, and face; stiffness or rigidity of the limbs and trunk; bradykinesia (slowness of movement); postural instability, or impaired balance and coordination.
- ✓ **Seizure**- Occurs when part(s) of the brain receives a burst of abnormal electrical signals that temporarily interrupts normal electrical brain function. (Howland and Mycek, 2006).

1.4. Definition of toxicity

Toxicity is defined as “the potential of a substance to exert a harmful effect on humans or animals, and a description of the effect and the conditions or concentration under which the effect takes place” (Health and safety, 2004). In order to support an application for a clinical trial or for the registration of a new drug, it is necessary to satisfy legislation that requires that certain data should be produced from a variety of toxicological investigations that show the safety profile of the compound to which humans may be exposed. Therefore, in the majority of cases of evaluation of the toxicity of most substances, rodents and non-human primates are first used in preclinical animal safety studies before further studies are done in humans. These animals are mainly used because of their biological similarity to humans that allows them to be regarded as the suitable metabolic models for humans in a broad range of investigations (Loomis and Hayes, 1996; Pascoe, 1983). In general, toxicity testing methods can be divided into two categories: The

first category comprises tests that are designed to evaluate the overall effects of compounds on experimental animals. Individual tests in this category differ from each other basically in regard to the duration of the test and the extent to which the animals are evaluated for general toxicity. These tests are classified as acute, prolonged and chronic toxicity tests (Loomis and Hayes, 1996). The second category of tests consists of those that are designed to evaluate specific types of toxicity in detail. The prolonged and chronic tests do not detect all forms of toxicity, but they may reveal some of the specific toxicities and indicate the need for more detailed studies. Thus, this second category of tests has been developed for the determination of effects of compounds on the fetus in a pregnant animal (teratogenic tests), on the reproductive capacity of the animals (reproduction hand, chronic effects are often detected over an extended period of time during which exposure may be continuous or intermittent, though obviously at levels which are too low to produce an acute effect (Loomis and Hayes, 1996; Pascoe, 1983).

1.4.1. Acute toxicity

Acute toxicity has been defined as “the ability of a substance to cause severe biological harm or death soon after a single exposure or dose for < 24 h; or any poisonous effect resulting from a single short-term exposure to a toxic substance”.

An acute toxicity test is a single test that is conducted in a suitable animal species and may be done for essentially all chemicals that are of any biologic interest. Its purpose is to determine the symptomatology consequent to administration of the compound and to determine the order of lethality of the compound. The test consists of administering the compound to the animals on one occasion (Loomis and Hayes, 1996; Timbrell, 2002).

1.4.2. Chronic toxicity

Chronic toxicity is defined as “the capacity of a substance to cause poisonous health effects in humans, animals, fish and other organisms after multiple exposures occurring over an extended period of time like > 3 months or over a significant fraction of an animal’s or human’s lifetime.

The purpose of the chronic toxicity test is to investigate the harmful effects that foreign compounds that are introduced to animals in repeated doses or in continuous exposure over an extended period of time may produce. The dose levels of compounds used usually range from a very low fraction of the therapeutically effective dose to doses that approach the maximum non-lethal dose (as established in rodent acute toxicity studies) (Poole and Leslie, 1989; Loomis and Hayes, 1996)

1.4.3. Toxic effects

Toxic effects are defined as “harmful responses of a biological system to a toxic compound, and death of cells or the whole organism are the major response” (Timbrell, 2002).

In all the cases, the toxic effects are usually manifested either in an acute or a chronic manner, and occur mostly as a result of an acute or chronic exposure to toxic compound by oral ingestion, inhalation or absorption following skin contact the toxic effects are seen as (1) signs or reflection of a disturbance of the normal activities of enzymes that perform essential biochemical roles in all forms of life; (2) alteration of the normal activities of plasma membrane that regulate the exchange of nutrients and metabolites between the cell and its surroundings and (3) the disturbances of other normal cell activities, e.g. RNA and DNA synthesis, growth, division and general metabolism at all levels of organization from sub-cellular to organ and organ system (Pascoe, 1983; Timbrell, 2002). The way in which the toxic agent is introduced into the body also plays significant role.

1.4.3.1 Routes of administration

This term refers to the way in which drugs or compounds are introduced to animal’s or humans. To evaluate toxicity of a compound in animals various routes may be used, but two most commonly used modes of administration for animals studies are via intra-peritoneal injection or the oral route (Poole and Leslie, 1989).

1.4.3.1.1 Intra-peritoneal injection

This is one of the methods of dosing, which may occasionally provide information about local as well as systemic toxicity. To give drugs by intra peritoneal dosing, the animal is laid on its back and the abdomen shaved. This area is thoroughly cleansed and, using an appropriate syringe and needle, the abdominal wall is punctured. To ensure minimal danger of perforation of abdominal viscera, the injection should be made rostral and lateral to the bladder at an angle of about 15° to the abdomen. The depth of penetration should not exceed 5 mm (Poole and Leslie, 1989; Waynforth, 1980).

1.4.3.1.2 Oral administration

The oral route is probably one of the most common means by which a chemical enters the body. In short, the oral administration is the form of administration involving the gastrointestinal tract, which may be viewed as a tube going through the body, starting at the mouth and ending at the anus. Although it is within the body, its contents are essentially exterior to the body fluids. Most orally administered chemicals can otherwise have a systemic effect on the organism only after absorption has occurred from the mouth or the gastrointestinal tract. Oral administration of chemicals that are rapidly absorbed from the gastrointestinal tract would theoretically expose the liver to concentrations of the agent that would not be obtained if other routes of administration were used (Loomis and Hayes, 1996). Furthermore, if a compound entered the enterohepatic cycle, at least a portion of the compound would be localized in the organs involved in the cycle. Compounds that are known to be toxic to the liver would be expected to be more toxic following oral administration on repeated occasions, whereas their administration by other routes may be less hazardous (Loomis and Hayes, 1996; Waynforth, 1980).

1.4.4. Hematology Introduction:

An analysis of blood was exercised from far back to ancient times. All three blood cell types performs its own role in healthy men's life and so count of different cell type of blood can identify different diseases that's the reason that complete blood cell count is the most common test carried out in all clinical laboratories. Different techniques were practiced since the discovery of blood cells in 1658. Before going into details of modern blood cell counting

methods we should know the history of cell counting and the developments in the technology of cell counting which was finally implemented to quantification of the ingredients of blood.

1.4.4.1 Hematology

In hematology we deal with the essentials of blood and the tissues for the forming blood. [Graham Ramsay *et al* 1999] Hematology is used to identify and examine the cure for anemia, leukemia's and hemophilia (a kind of blood disease). Hematological tests are performed to check the results of certain treatments e.g. cancer chemotherapy and also to get outcome about the patients overall health.

1.4.4.2 History of Cell counting

Leeuwenhoek was the first person who attempted to count blood cells using a glass capillary tube with graduation marks of measured dimension and microscope to count. He selected chicken to count red blood cells [Hajdu, SI 1998;42:1075]. Afterwards, different techniques were introduced for diluting the blood which resulted in more accurate and easier counting using a shallow rectangular chamber which had a thin cover glass and diluted blood was injected into this glass. In the early 20th century a technique using photoelectric device to count cells was invented by Moldovan [Bennett, 1841.] However, this attempt for cell counting did not develop at that time because of the unreliability of the photoelectric device. An automated blood-cell counter technique was invented by Waiter H. Coulter [Hajdu, SI 1998;42:1075] in the mid 1950's for blood cell counting. The research was based on the technique known as "Coulter's Principle" or the Aperture Impedance technique. This technique uses the resistivity of the blood cells because the impedance of the cells suspended in the diluting fluid is much more higher than that of fluid was based on the fact that the resistivity of blood cells is much higher than that of the diluting fluid. Most modern cell counters serves on the basis of this extensively developed since 1950's

1.4.5. Cellular Elements of Blood

Blood is a circulating tissue composed of fluid plasma and cells (red blood cells, white blood cells, platelets). Anatomically, blood is considered a connective tissue, due to its origin in the bones and its function. Blood is the means and transport system of the body used in carrying

elements (e.g. nutrition, waste, heat) from one location in the body to another, by way of blood vessels.

Blood is made of two parts:

1. Plasma which makes up 55% of blood volume.
2. Formed cellular elements (red and white blood cells, and platelets) which combine to make the remaining 45% of blood volume (Alberts, 2012).

1.4.5.1 Plasma

Plasma is made up of 90% water, 7-8% soluble proteins (albumin maintains blood's osmotic integrity, others clot, etc), 1% carbon-dioxide, and 1% elements in transit. One percent of the plasma is salt, which helps with the pH of the blood. The largest group of solutes in plasma contains three important proteins to be discussed. There are: albumins, globulins, and clotting proteins. Plasma also carries Respiratory gases; CO₂ in large amounts (about 97%) and O₂ in small amounts (about 3%), various nutrients (glucose, fats), wastes of metabolic exchange (urea, ammonia), hormones, and vitamins.

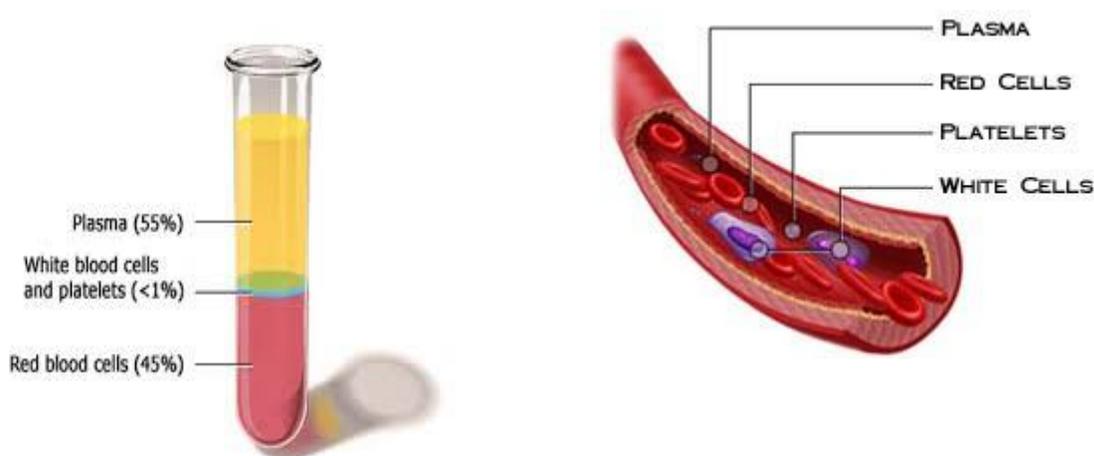


Figure 1.7: Plasma of the Blood

Red Blood cell (*Erythrocytes*):

Erythrocytes are the most important and major elements of blood. There are normally 4-6 million in number in a normal human body. Hemoglobin a major part of RBCs, carry oxygen from the lungs to the tissues and carbon dioxide from the tissues back to the lungs. If any variation in

RBCs count is found, it can result in many symptoms and diseases can attack on an individual. So RBCs play an important role in identifying a variety of disease.

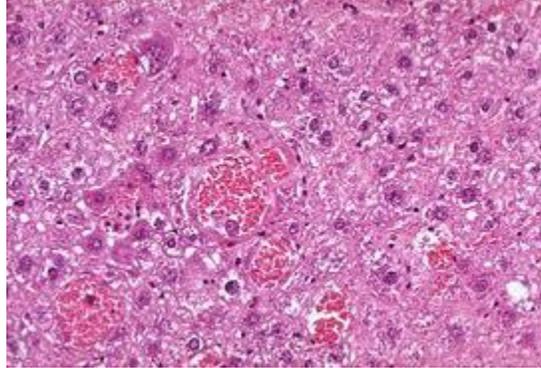


Figure 1.8: Red Blood cell

- **Normal range of RBC $8-16 \times 10^6 \text{mm}^3$**

1.4.6. Different count of RBC

Hemoglobin: Hemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates as well as the tissues of some invertebrates. Hemoglobin in the blood carries oxygen from the respiratory organs (lungs or gills) to the rest of the body (i.e. the tissues) where it releases the oxygen to burn nutrients to provide energy to power the functions of the organism in the process called metabolism.

Role in disease

- Hemoglobin deficiency can be caused either by decreased amount of hemoglobin molecules, as in anemia (Anemia is a decrease in number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood), or by decreased ability of each molecule to bind oxygen at the same partial pressure of oxygen.
- hemoglobin deficiency decreases blood oxygen-carrying capacity
- Other common causes of low hemoglobin include loss of blood, nutritional deficiency, bone marrow problems, chemotherapy, kidney failure, or abnormal hemoglobin

Pharmacological Study Of *Phyla Nodiflora*

- High hemoglobin levels may be caused by exposure to high altitudes, smoking, dehydration, or tumors
- Elevated levels of hemoglobin are associated with increased numbers or sizes of red blood cells, called polycythemia. (Polycythemia is a disease state in which the proportion of blood volume that is occupied by red blood cells increases. Blood volume proportions can be measured as hematocrit level. It can be due to an increase in the number of red blood cells or to a decrease in the volume of plasma . Polycythemia is sometimes called erythrocytosis)

Hematocrit: The hematocrit also known as packed cell volume (PCV) is the volume percentage (%) of red blood cells in blood. It is normally about 45% for men and 40% for women. It is considered an integral part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count, and platelet count.

Higher than Normal Hematocrit

- In cases of dengue fever, a high hematocrit is a danger sign of an increased risk of dengue shock syndrome.
- Polycythemia vera (PV), a myeloproliferative disorder in which the bone marrow produces excessive numbers of red cells, is associated with elevated hematocrit.
- Chronic obstructive pulmonary disease (COPD) and other pulmonary conditions associated with hypoxia may elicit an increased production of red blood cells. This increase is mediated by the increased levels of erythropoietin by the kidneys in response to hypoxia.
- Anabolic androgenic steroid (AAS) use can also increase the amount of RBCs and, therefore, impact the hematocrit, in particular the compounds boldenone and oxymetholone.
- If a patient is dehydrated, the hematocrit may be elevated.
- Capillary leak syndrome also leads to abnormally high hematocrit counts, because of the episodic leakage of plasma out of the circulatory system.
- Sleep apnea has been known to cause elevated hematocrit levels.

Lower than Normal Hematocrit

- Infants without adequate iron intake

Pharmacological Study Of *Phyla Nodiflora*

- children going through a rapid growth spurt, during which the iron available cannot keep up with the demands for a growing red cell mass
- menstruating women, who have a greater need for iron because of blood loss during menstruation
- pregnant women, in whom the growing fetus creates a high demand for iron
- Patients with chronic kidney disease whose kidneys no longer secrete sufficient levels of the hormone erythropoietin that promotes RBC proliferation. Erythropoietin prevents the death of cells in the erythrocyte cell line in the bone marrow. Therefore, erythropoietin allows those cells to continue to mature, exit the bone marrow and become RBCs (Jelkmann, 2004).

Mean corpuscular volume, or mean cell volume (MCV)

The mean corpuscular volume, or mean cell volume (MCV), is a measure of the average volume of a red blood corpuscle (or red blood cell). The measure is attained by multiplying a volume of blood by the proportion of blood that is cellular (the hematocrit or haematocrit), and dividing that product by the number of erythrocytes (red blood cells) in that volume. The mean corpuscular volume is a part of a standard complete blood count. In a laboratory test that computes MCV, erythrocytes are compacted during centrifugation. The normal reference range is typically 80-100 fL.

Higher than Normal MCV

- In pernicious anemia (macrocytic), MCV can range up to 150 femtolitres.
- An elevated MCV is also associated with alcoholism (as are an elevated GGT and a ratio of AST:ALT of 2:1).
- Vitamin B12 and/or folic acid deficiency has also been associated with macrocytic anemia (high MCV numbers).

Lower than Normal MCV

- The most common causes of microcytic anemia are iron deficiency (due to inadequate dietary intake, gastrointestinal blood loss, or menstrual blood loss), thalassemia, sideroblastic anemia or chronic disease. In iron deficiency anemia (microcytic anemia), it can be as low as 60 to 70 femtolitres.

- In some cases of thalassemia, the MCV may be low even though the patient is not iron deficient (Tonnesen, 1986).

Mean corpuscular hemoglobin (MCH)

The mean corpuscular hemoglobin (MCH), or "mean cell hemoglobin" (MCH), is the average mass of hemoglobin per red blood cell in a sample of blood. It is reported as part of a standard complete blood count. MCH value is diminished in hypochromic anemias. It is calculated by dividing the total mass of hemoglobin by the number of red blood cells in a volume of blood. $MCH = (Hgb * 10) / RBC$. A normal value in humans is 27 to 31 picograms/cell.

Higher than Normal MCH

Generally, if the MCH level is over 34, this is considered to be too high. The main reason that the MCH level would be too high is because of macrocytic anemia.

- Macrocytic anemia is a blood disorder in which not enough red blood cells are produced, but the ones that are present are large (thus fitting more hemoglobin).
- Macrocytic anemia is often caused by having too little vitamin B12 or folic acid (a type of vitamin) in the body.

Lower than Normal MCV

Generally, if the MCH level is below 26, this is considered too low. The MCH level can be too low because of

- blood loss over time,
- too little iron in the body,
- or Microcytic anemia which is a condition in which abnormally small red blood cells are present. Smaller red blood cells means that less hemoglobin fits in each cell.
- Hemoglobinopathy, which is a group of disorders characterized by changes in the structure of hemoglobin, can also cause a low MCH level.

Mean corpuscular hemoglobin concentration (MCHC)

Mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin per unit volume of red blood cells and is calculated by dividing the hemoglobin by the hematocrit.

$$\text{MCHC} = \text{H}_b / \text{H}_{ct} \times 100$$

Normal range: 32-36 g/dL

When the MCHC is abnormally low they are called hypochromic, and when the MCHC is abnormally high, hyperchromic.

Red blood cell distribution width (RDW or RCDW)

Red blood cell distribution width (RDW or RCDW) is a measure of the variation of red blood cell (RBC) volume that is reported as part of a standard complete blood count. Usually red blood cells are a standard size of about 6-8 μm in diameter. Certain disorders, however, cause a significant variation in cell size. Higher RDW values indicate greater variation in size. Normal reference range in human red blood cells is 11.5-14.5%. If anemia is observed, RDW test results are often used together with mean corpuscular volume (MCV) results to determine the possible causes of the anemia. It is mainly used to differentiate an anemia of mixed causes from an anemia of a single cause.

Higher than Normal RDW

- Iron Deficiency Anemia: usually presents with high RDW with low MCV
- Folate and vitamin B12 deficiency anemia: usually presents with high RDW and high MCV
- Mixed Deficiency (Iron + B12 or folate) anemia: usually presents with high RDW with MCV being high, low or often normal range
- Recent Hemorrhage: typical presentation is high RDW with normal MCV
- A false high RDW reading can occur if EDTA anticoagulated blood is used instead of citrated blood.

1.4.7. White Blood Cell

WBCs are the minor part of blood cells as their count is 9,000 – 30,000 / mm³ for a newly born and after few weeks it decreases to 6,000 – 11,000 / mm³. An adult has only 4,000 – 11,000 / mm³ of leukocytes. WBCs consist of neutrophils, basophiles, eosinophiles, monocytes and lymphocytes. The lymphocytes control the immune system of human body and fight against the harmful germs in the body. Lymphocytes produce antibodies. Lymphocytes increase their number when a viral infection takes place. Neutrophils play a defensive role in attacking germs and harmful bodies. They also increase when bacterial infection is found in the body. The WBCs have a variety of life spans, some live few days and the others last for several of months. Leukocytes live in tissues and other parts of body but just use blood as a mean of transportation

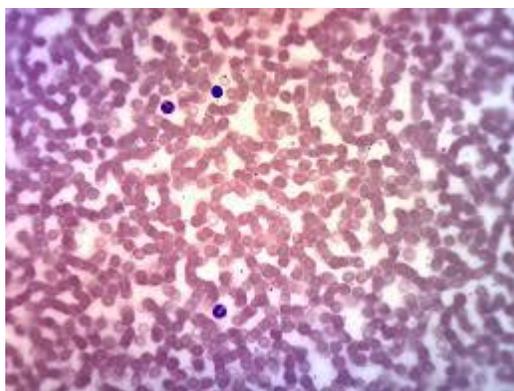


Figure 1.9: White Blood Cells

- **Normal range of WBC: $3-7 \times 10^3 \text{mm}^3$**

Different count of WBC

Neutrophils: Approx 70%, it is responsible for providing the body with a defense against invading micro organisms. It ingests & kills the organisms by digesting them, a process known as phagocytosis.

Eosinophils: Approx 4%, they also help in destroying organism.

Basophils: Apporx 1%, they release histamine, thus helping in hypersensitivity reaction.

Lymphocytes: About 23%, it is the key element in producing immunity.

Monocytes: About 2%, they engulf foreign particles & destroy them.

1.4.8. Platelets (Thrombocytes):

Platelets are fragments of cytoplasm that are fired out in the blood from large cells in the bone marrow. So some physicians don't consider them complete blood cells. Platelets work importantly in blood clotting known as haemostasis. Vessel walls are surrounded by platelets to stop bleeding when injured. They also help in infections from enzymatic reactions. Normal range of platelet: $1000-1600 \times 10^3 \text{mm}^3$ (Ganong, 2003)

- **Normal range of platelet: $1000-1600 \times 10^3 \text{mm}^3$**

1.4.9. Hepatotoxicity

Hepatotoxicity The liver's status as the largest organ in the body reflects its key roles in many physiological processes, ensuring its undisputed position as 'metabolic coordinator' of the entire body. Due to the organ's importance to many body functions, any tendency for a chemical to damage the liver is taken very seriously in modern toxicology and risk assessment.

Several factors predispose the liver to xenobiotic toxicity.

- Firstly, for chemicals entering the body via the oral route, anatomical proximity to the GI-tract ensures the liver is the 'first port of call' for ingested xenobiotics.
- Secondly, chemicals and nutrients are not the only substances that enter portal blood as it perfuses the intestines: it also accumulates products of the degradation of intestinal microorganisms such as inflammogenic lipopolysaccharide components of the bacterial cell wall (i.e. endotoxin). Since endotoxin delivery may increase during xenobiotic intoxication, immunological responses to co-absorbed endotoxin can exacerbate the hepato-toxicity of ingested chemicals.
- Thirdly, in addition to entry via the portal circulation, chemicals can access the liver via arterial blood that mixes with venous blood in the hepatic sinusoids. For example, inhaled tobacco constituents that enter via the lungs are efficiently delivered to the liver via the arterial route.

- Fourthly, the vast metabolic capacities of the liver also paradoxically heighten its vulnerability to chemical toxicity: by functioning as a miniaturised chemical factory that performs many diverse chemical modifications on foreign molecules, CYPs and other hepatic enzymes can inadvertently generate noxious metabolites that induce ‘bioactivation-dependent’ hepatotoxicity (Philip, and Burcham, 2014).

1.4.10. Liver

The liver is a vital organ present in vertebrates and some other animals. It has a wide range of functions, including detoxification, protein synthesis, and production of biochemicals necessary for digestion. The liver is necessary for survival; there is currently no way to compensate for the absence of liver function in the long term, although new liver dialysis techniques can be used in the short term.

This gland plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It lies below the diaphragm in the abdominal-pelvic region of the abdomen. It produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions.



Figure 1.10: Liver

Anatomy

The liver is a reddish brown organ with four lobes of unequal size and shape. A human liver normally weighs 1.44–1.66 kg (3.2–3.7 lb), and is a soft, pinkish-brown, triangular organ. It is both the largest internal organ (the skin being the largest organ overall) and the largest gland in the human body. It is located in the right upper quadrant of the abdominal cavity, resting just below the diaphragm. The liver lies to the right of the stomach and overlies the gallbladder. It is connected to two large blood vessels, one called the hepatic artery and one called the portal vein. The hepatic artery carries blood from the aorta, whereas the portal vein carries blood containing digested nutrients from the entire gastrointestinal tract and also from the spleen and pancreas. These blood vessels subdivide into capillaries, which then lead to a lobule. Each lobule is made up of millions of hepatic cells which are the basic metabolic cells. Lobules are the functional units of the liver.

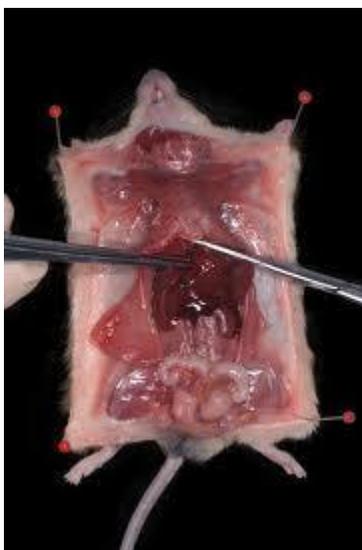


Figure1.11: Anatomy of mice

1.4.10.1. Liver function tests

Liver function tests (LFTs or LFs) are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver.(Lee, Mary (2009-03-10)The parameters measured include prothrombin time (PT/INR), aPTT, albumin, bilirubin (direct and indirect), and others. Liver transaminases (AST or SGOT and ALT or SGPT) are useful

Pharmacological Study Of *Phyla Nodiflora*

biomarkers of liver injury in a patient with some degree of intact liver function. (Johnston, David (15 April 1999). Most liver diseases cause only mild symptoms initially, but these diseases must be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. This testing is performed by a medical technologist on a patient's serum or plasma sample obtained by phlebotomy. Some tests are associated with functionality (e.g., albumin), some with cellular integrity (e.g., transaminase), and some with conditions linked to the biliary tract (gamma-glutamyltransferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to detect the presence of liver disease, distinguish among different types of liver disorders, gauge the extent of known liver damage, and follow the response to treatment. Some or all of these measurements are also carried out (usually about twice a year for routine cases) on those individuals taking certain medications — anticonvulsants are a notable example — to ensure the medications are not damaging the mice's liver.

Albumin:

Albumin is a protein made specifically by the liver, and can be measured cheaply and easily. It is the main constituent of total protein (the remaining from globulins). Albumin levels are decreased in chronic liver disease, such as cirrhosis. It is also decreased in nephrotic syndrome, where it is lost through the urine. The consequence of low albumin can be edema since the intravascular oncotic pressure becomes lower than the extravascular space. An alternative to albumin measurement is prealbumin, which is better at detecting acute changes (half-life of albumin and prealbumin is about 2 weeks and about 2 days, respectively)

Aspartate transaminase:

AST, also called serum glutamic oxaloacetic transaminase or aspartate aminotransferase, is similar to ALT in that it is another enzyme associated with liver parenchymal cells. It is raised in acute liver damage, but is also present in red blood cells, and cardiac and skeletal muscle, so is not specific to the liver. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage. (Nyblom H *et al.*, Alcohol. 39 (4): 336–339) Elevated AST levels are not specific for liver damage, and AST has also been used as a cardiac marker.

SGPT test:

This test measures the amount of an enzyme called glutamate pyruvate transaminase (GPT) in blood. This enzyme is found in many body tissues in small amounts, but it is very concentrated in the liver. It is released into the blood when cells that contain it are damaged. This enzyme is also called alanine transaminase, or ALT.

- **The normal ranges of SGPT in mice 330U/ml apparently (borderline range, 30-380 U/ml) .**

SGPT levels may be higher than normal also if:

- drink too much alcohol.
- mononucleosis.
- chronic liver infection or inflammation.
- gallbladder inflammation, such as may be caused by gallstones.
- a gallbladder infection.
- congested blood flow through the liver due to heart failure.
- liver cancer or another cancer that has spread to the liver.
- taking certain medicines, such as:
 - `medicines used to lower cholesterol levels
 - antifungal medicines
 - some narcotics and barbiturates
 - methotrexate
 - acetaminophen
 - salicylates (aspirin)

Transaminases:

AST/ALT elevations instead of ALP elevations favor liver cell necrosis as a mechanism over cholestasis. When AST and ALT are both over 1000 IU/L, the differential can include acetaminophen toxicity, shock, or fulminant liver failure. When AST and ALT are greater than three times normal but not greater than 1000 IU/L, the differential can include alcohol toxicity, viral hepatitis, drug-induced level, liver cancer, sepsis, Wilson's disease, post-transplant rejection of liver, autoimmune hepatitis, and steatohepatitis (nonalcoholic). AST/ALT levels elevated minorly may be due to rhabdomyolysis, among many possibilities.

Alkaline phosphatase:

Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. ALP levels in plasma rise with large bile duct obstruction, intrahepatic cholestasis, or infiltrative diseases of the liver. ALP is also present in bone and placental tissue, so it is higher in growing children (as their bones are being remodeled) and elderly patients with Paget's disease. In the third trimester of pregnancy, ALP is about two to three times higher.

ALP - blood test

Alkaline phosphatase (ALP) is a protein found in all body tissues. Tissues with higher amounts of ALP include the liver, bile ducts, and bone.

Normal Range:

The the range of activity for 306 apparently normal adult mice was **10–210 mU/ml** (international milliunits/ml), with a mean of 67 and a standard error of 1.7.

Normal values may vary slightly from laboratory to laboratory. They also can vary with age and gender. High levels of ALP are normally seen in little mice undergoing growth spurts and in pregnant mice.

The examples above show the common measurements for results for these tests. Some laboratories use different measurements or may test different specimens.

Higher-than-normal ALP levels

- Biliary obstruction
- Bone conditions
- Osteoblastic bone tumors, osteomalacia, a fracture that is healing
- Liver disease or hepatitis
- Eating a fatty meal if you have blood type O or B
- Hyperparathyroidism
- Leukemia
- Lymphoma
- Paget's disease
- Rickets
- Sarcoidosis

Lower-than-normal ALP levels

- Hypophosphatasia
- Malnutrition
- Protein deficiency
- Wilson's disease

Other conditions for which the test may be done:

- Alcoholic liver disease (hepatitis/cirrhosis)
- Alcoholism
- Biliary stricture
- Gallstones
- Giant cell (temporal, cranial) arteritis
- Multiple endocrine neoplasia (MEN) II
- Pancreatitis
- Renal cell carcinoma

Chapter-2

Plant Introduction

2. Introduction to Plant

2.1. Plant information:

A prostrate, much branched annual herb, often rooting at the nodes, up to 75 cm long. Leaves cuneate-spathulate, serrate, 2.5 cm long. Peduncles commonly axillary; heads 1.25 cm long, ovoid or cylindrical. Flowers small, pinkish-purple to white.

2.1.1. Scientific name: *Phyla nodiflora*

2.1.2 Local Name:

- Bhui Okar
- Okra pata
- Saitta okra
- Karghas
- Bakkan

2.1.3 Common name:

- Turkey tangle fogfruit

2.1.4 Synonyms :

- *Lippia incisa* (Small) Tidestr.
- *Lippia nodiflora* (L.) Michx.
- *Lippia nodiflora* (L.) Michx. var. *reptans* (Kunth) Kuntze
- *Phyla nodiflora* (L.) Greene var. *incisa* (Small) Moldenke

2.1.5 Taxonomic position:

Kingdom	Plantae – Plants
Subkingdom	Tracheobionta - Vascular plants
Superdivision	Spermatophyta - Seed plants
Division	Magnoliophyta - Flowering plants
Class	Magnoliopsida – Dicotyledons
Subclass	Asteridae
Order	Lamiales
Family	Verbenaceae - Verbena family
Genus	<i>Phyla</i> Lour. – fogfruit
Species	<i>Phyla nodiflora</i> (L.) Greene - turkey tangle fogfruit

2.2. Description:

A prostrate, much branched annual herb, often rooting at the nodes, up to 75 cm long. Leaves cuneate-spathulate, serrate, 2.5 cm long. Peduncles commonly axillary; heads 1.25 cm long, ovoid or cylindrical. Flowers small, pinkish-purple to white.



Figure 2.1: *Phyla nodiflora* (L.)

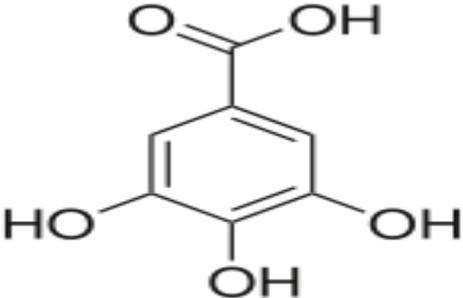
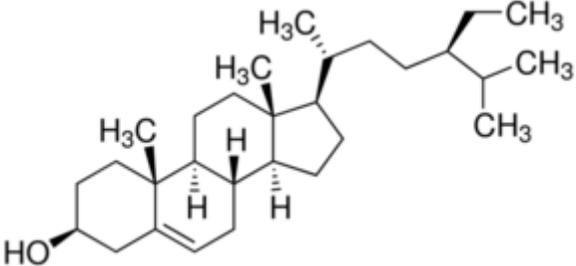
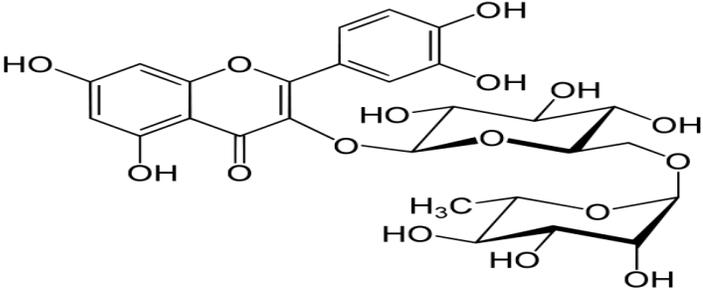
2.3. Geographical Distribution:

Throughout the country in fallow lands.

2.4. Chemical composition:

Leaves contain tannin, fat, lactose, maltose, glucose, fructose, xylose, rutin, a waxy ester, β -sitosterol, a bitter substance and two glucosidic colouring matters, nodiflorin A and B. Nodifloridin A and B, nodifloretin, two new flavone glycoside - lippiflorin A and B have also been isolated from leaves (Ghani, 2003, Rastogi and Mehrotra, 1990 & 93).

Pharmacological Study Of *Phyla Nodiflora*

Type	Compound	Structure
Tannin	$C_7H_{13}O$	 <p style="text-align: center;">Gallic acid</p>
β -sitosterol	$C_{29}H_{50}O$	 <p style="text-align: center;">β-sitosterol</p>
Rutin	$C_{27}H_{30}O_{16}$	 <p style="text-align: center;">Rutin</p>

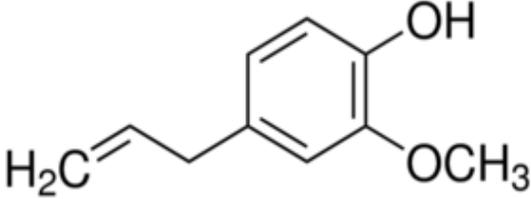
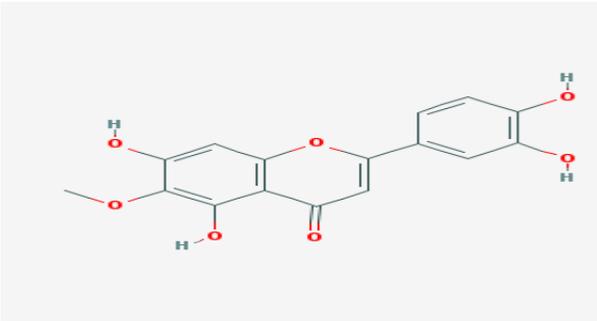
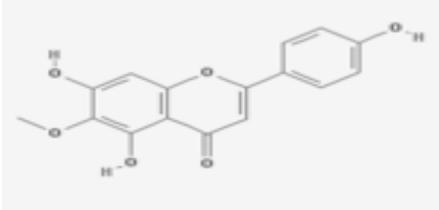
Eugenol	$C_{10}H_{12}O_2$	 <p>Eugenol</p>
6-Methoxyluteolin	$C_{16}H_{12}O_7$	 <p>6-Methoxyluteolin</p>
Hispidulin	$C_{16}H_{12}O_6$	 <p>Hispidulin</p>

Figure 2.2: Chemical composition of *Phyla nodiflora* (L.)

2.5. Medicinal Uses:

Pain due to working or falling down, pain in back or waist due to rheumatism. 2 tolas (1/2 cup) amount of leaf juice are taken once for pain due to working or falling down. The leaves, stems and barks of *Phyla nodiflora* is combined with leaves, stems and barks of *Solanum capsicoides* and crushed to extract the juice. 1/2 cup amount of juice is taken in the morning and evening on an empty stomach for rheumatic pain. (Chowdhury, 2010)

The plant is diuretic, stomachic, febrifuge and astringent to the bowels; good for ulcers, wounds, asthma, bronchitis; considered valuable in ischury, stoppage of the bowels and pain in the knee-joints. A poultice composed of the fresh plant is a good maturant for boils. Infusion of the leaves and tender stalks is given to children suffering from indigestion and to women after delivery. Chutney made from the leaves and fruits are eaten to relieve the irritation of internal piles

Chapter-3

Literature Review

3. Review of literature:

3.1. Antibacterial activity:

The ethanol extract of whole plant of *Phyla nodiflora* and its sub fractions (n-hexane, chloroform, ethyl acetate, n-butanol and aqueous) were subjected to antibacterial assay against seven bacterial strains i.e. *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus epidermidis*, *Staphylococcus aureus* and *Bacillus subtilis*. Ethyl acetate and chloroform fractions at concentration of 20 mg/ml showed excellent activity against *Bacillus subtilis*, *Staphylococcus epidermidis* and *Staphylococcus aureus*. Ethyl acetate fraction showed maximum zone of inhibition (16mm) for *Salmonella typhi* and chloroform fraction showed maximum zone of inhibition (18mm) for *Staphylococcus aureus*. The details of antibacterial inhibition are shown in Table-1.(Zakir et al., 2013). The methanol extracts of seeds of *Phyla nodiflora* exhibits antibacterial activity against *Bacillus subtilis*, *Styphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Patel et al., 2011). Hexane, chloroform and alcohol extracts of leaf of *Phyla nodiflora* were evaluated for antibacterial activity against *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Shigella flexneri* and *Salmonella Paratyphi A*. The chloroform extract showed maximum zone of inhibition (13mm) for *Bacillus subtilis* and alcohol extract showed maximum zone of inhibition against *Bacillus cereus* (11mm).

3.2. Antifungal activity:

The ethyl acetate, ethanol, methanol and water extracts of whole plant of *Phyla nodiflora* had been screened for antifungal activity against *Aspergillus niger*, *Aspergillus flavus*, *Paecilomyces varioti*, *Microsporum gypseum*, *Trichophyton rubrum*. Ethanol extract showed 100 % inhibition against tested organism as compared to aqueous (82.6%), methanol (61 %), ethyl acetate (87 %) extracts (Pascual et al., 2001; Pirzada et al., 2005)

3.3. Antidiabetic and hypolipidemic effect:

γ – sitosterol isolated from methanol extract of *Lippia nodiflora* was screened for antidiabetic potential. γ - sitosterol at 20 mg/kg body weight was administered to streptozotocin induced diabetic rats. A decrease in glycosylated hemoglobin and blood glucose level with significant increase in plasma insulin, body weight and food intake were observed. Furthermore γ -sitosterol had also been evaluated for antihyperlipidemic effect. The compound was responsible for

decreasing serum cholesterol, very low density lipoprotein and triglycerides levels with elevated level of high density lipoprotein which indicated its hepatoprotective effect (Rangachari and Savarimuthu 2011).

3.4. Anti-inflammatory activity:

Methanol extract of whole plant of *Lippia nodiflora* also exhibited anti-inflammatory and nociceptive activities at 20 µg/ml. Cyclo-pentano phenatherol isolated from methanol extract of *Lippia nodiflora* showed inhibition of COX-2 and prostaglandin biosynthesis at 10 µg/ml and thus, it was concluded that *Phyla nodiflora* have potential to cure inflammation (Durairaj et al., 2007; Ahmad et al., 2004).

3.5. Neuropharmacological effects:

Petroleum ether, chloroform and methanol extracts of aerial parts of *Phyla nodiflora* were evaluated for neuropharmacological effects. Diazepam was used as standard drug to monitor the parameters like sleeping time, locomotor activity, exploratory behavior pattern, motor coordination and convulsions. Flavonoids present in ethanol and chloroform extract were found to be responsible anticonvulsants for central inhibitory, and anxiolytic effects at 500mg/kg. Petroleum ether extract showed absence of flavonoids thus did not produce any central inhibitory effect (Kumaresan et al., 2011).

3.6. Antiurolithiatic activity:

The ethanol extract of *Phyla nodiflora* was studied for investigation of antiurolithiatic activity against most common calcium oxalate type of kidney stone. Gentamycin and calculi producing diet was administered for producing calcium oxalate urolithiasis. The extract was also accessed for in vivo antioxidant parameters like catalase, lipid peroxidation and reduced glutathione and in vitro scavenging of nitric oxide and free radicals that helps in preventing calcium oxalate type stone formation and dissolving of preformed stones in kidney. The extract was found to be safe up to maximum dose of 8g/kg (Dodala et al., 2010).

3.7. Hepatoprotective and antioxidant potential:

The methanol extract of whole plant of *Lippia nodiflora* has been evaluated for antioxidant activity and hepatoprotective effects in paracetamol induced liver injury (750mg/kg, body weight). The extract was orally administered for 7 days. A significant decrease in the level of blood serum enzymes such as serum glutamicoxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), aspartate transaminase (ALP), bilirubin and lipid peroxidation was observed. The hepatoprotective effect of *Lippia nodiflora* was found to be equivalent to that of standard silymarin 25mg/kg (Mazumde et al., 2008).

3.8. Antiproliferative and apoptotic effect:

The methanol and ethyl acetate extracts of leaves and stems of *Phyla nodiflora* have antiproliferative and apoptotic effects on human breast cancer cell line. The extracts at 90-120µg/ml were capable of inhibiting cancer cell growth via apoptosis (Rabi'atul'adawiyah et al., 2014)

3.9. Diuretic activity:

Diuretic potential of methanol and aqueous extracts of dried aerial parts of *Phyla nodiflora* was determined using in-vivo Lipschitz test model in albino rats. Furosemide was used as a standard to measure the parameters like increase in urine volume and electrolyte excretion. Both extracts have significant diuretic activity at 500mg/kg body weight (Shukla et al., 2009).

3.10. Cytotoxic activity:

Halleridone and Halleron isolated from dichloromethane and methanol extracts of the leaves of *Phyla nodiflora* are known to have anticancer, antitumor and cytotoxic activities (Ravikanth et al., 2000). The methanol extract of whole plant of *Phyla nodiflora* was evaluated for antitumor effect using Erich's ascites carcinoma (EAC) in Swiss albino mice at 200 and 400 mg/ kg of body weight. The extract was found to decrease tumor cell volume, cell count and packed cell volume. The hematological parameters did not significantly altered at these specified doses but the levels of catalase, reduced glutathione and superoxide dismutase were increased and lipid peroxidation reduced which indicated that the anti-tumor activity of methanol extract of whole

plant of *Phyla nodiflora* was due to the presence of antioxidant components (Durairaj et al., 2009).

3.11. Anti -melanogenesis activity:

Eupafolin isolated from methanol extract of dried aerial parts of *Phyla nodiflora* is responsible for inhibiting tyrosine activity to down regulate melanogenesis in a dose dependent manner (0.110 μ M) (Ko et al., 2013 and Yen et al., 2012). Hyperpigmentation is caused by overproduction of tyrosinases enzymes including TYR, TRP-1 and TRP-2 which leads to melasma (Hearing and Tsukamoto 1991).

Chapter-4

Materials and Methods

4.1. Plant Materials

4.1.1 Collection of plant

The plant was collected from Magura district of Bangladesh. *Phyla nodiflora* contains variety of phytochemical constituents such as *Phyla nodiflora*, *Lippia nodiflora*, *Lippia incisa* and *Phyla incisa*. *Phyla nodiflora* is known by the local people as Jal papli, Lippia, Frog fruit and Bukkhan. *Phyla nodiflora* is fast growing perennial prostrate herb. Leaves; obovate, obtuse, somewhat fleshy, and rarely subacute. Their surface is covered with fine hairs and color is as alkaloids, glycosides, flavonoids tannins, phenolic compounds, steroids, terpenoids, carbohydrates, proteins, amino acids, gums and mucilage. Flavonoids are present as major component. The proper time of harvesting or collecting is particularly important because the nature and the quantity of constituents vary gently in some species according to the season.

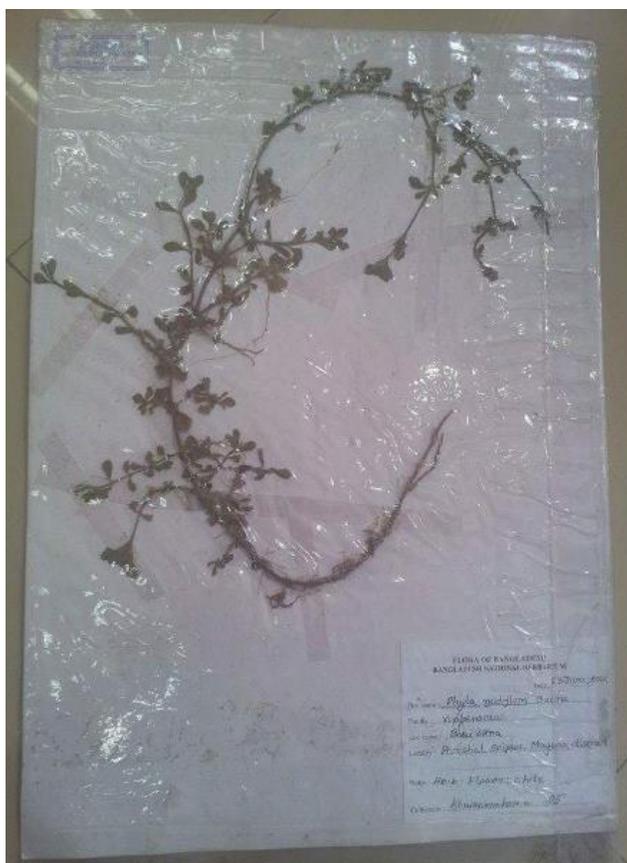


Figure 4.1. Herbarium sheet of *Phyla nodiflora*

4.1.2. Preparation of plant extraction

The whole part of the plant was dried in room temperature for approximately two weeks. Then the dried plants were taken into fine powder by using a grinding machine. Then the extraction process was done.

At first 2kg dried plant dust of *Phyla nodiflora* was soaked in 8L methanol in four bottles. Then it was kept in room temperature for 3 days and everyday it was used to shake properly to ensure the maximum amount of constituents present in the grinded plant become soluble into methanol. After 3 days later, the mixture was filtered. For filtration, white cotton cloth was used. After filtration two parts were obtained.

1. The residue portion over the filter
2. The filtered part

The filtrated part, which contains the substance soluble in methanol, poured into a 1000 round bottle flask, and then the flask was placed in a rotary evaporator. The evaporation was done at 50°C temperature. The number of rotation per minute was selected as 100 RPM. The pressure of vacuum pump machine was 6 bars. The water flow through the distillation chamber was also provided in a satisfactory flow rate.

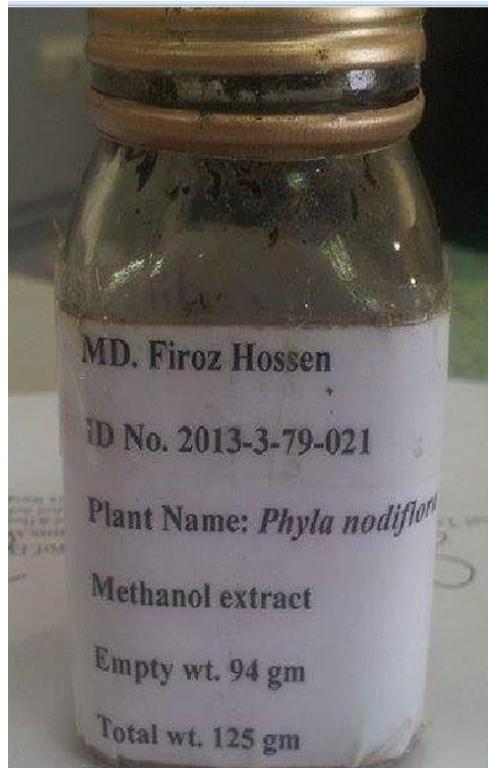


Figure 4.2: Rotary evaporator & crude extract in a bottle

4.2. Experimental Animals

Swiss mice of either sex (20-25gm) were obtained from the Animal house of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55-65%, r.t. $23.0 \pm 2.0^\circ\text{C}$ and 12 h light: dark cycle). The animals were fed with standard diet and water ad libitum.



Figure 4.3 : *Swiss albino Mice*

4.3. Equipments

Spatula, mortar and pestle, large beaker (1000 ml), small beaker (50ml), pipette, filter paper (Whatman 40), vial (5ml), mice oral needle, 1m insulin syringe (50 units), petri dishes, distilled water, forceps, Scissors, masking tape, permanent marking pen, aluminium foil paper, test tube, analytical balance (ELH 3000, Shimadzu, Japan), refrigerator, pencil, scale, container.

4.4. Drugs and Chemicals

4.4.1. Chemical Agents

1. 5% CMC (Vehicle) 10ml/kg as negative control,
2. 0.3 mL of charcoal meal of distilled water suspension containing 10% gum acacia, 10% activated charcoal and 20% starch.

4.4.2. Standard Drug

1. Bisacodyl (5mg/kg, p.o.) used as positive control
2. Atropine (10 mg/kg, i.p.)

4.5. Evaluation of Laxative activity

4.5.1. Charcoal meal GI transit test

The method described by (Al-Qarawi et al., 2003) was followed with slight modifications. Mice fasted for 12 h were divided into 8 different groups (5 animals in each). Three of the groups were

Pharmacological Study Of *Phyla Nodiflora*

treated per oral (p.o.) with increasing doses of 200mg/kg, 400 mg/kg & 800mg/kg of the plant *Phyla nodiflora* acting as the test groups. One group was taken as negative control, treated with 5%CMC (10 mL/kg). The next group was administered Bisacodyl (5 mg/kg) as the positive control. After 15 min, the animals were given 0.3 mL of charcoal meal of distilled water suspension containing 10% gum acacia, and 20% starch. The animals were sacrificed after 30 min and the abdomen was opened to excise the whole small intestine. The length of the small intestine and the distance between the pylorus region and the front of the charcoal meal was measured to obtain the charcoal transport ratio or percentage. In order to assess the involvement of acetylcholine (ACh)-like prokinetic effect of the extract, further groups of mice were pretreated with intraperitoneal (i.p.) injection of atropine (10 mg/kg) 15 min prior the administration of the extract.



Dose: Negative



Dose: 200mg/kg



Dose: 400mg/kg

Pharmacological Study Of *Phyla Nodiflora*



Dose: 800mg/kg



Dose: Positive



Dose: 400mg/kg + Atropine



Dose: 800mg/kg + Atropine



Dose: Positive + Atropine

Figure4.4: GI transit into mouse

4.6. CNS Activity Test

4.6.1. Materials for CNS Activity Test:

- Analytical Balance,
- Feeding needle: 1 c.c.
- Insulin syringes 100 units both disposable and nondisposable
- Open Field Board
- Hole board
- Lamp light
- Stop Watch

4.6.2. Chemical Agents Used in CNS activity Test:

- 5% CMC (Vehicle) 10ml/kg as negative control,

4.6.3. Standard Drugs Used in CNS activity Test:

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

4.6.4. Doses Used in CNS Activity Test of the Extract:

4.6.4.1 Open Field Test:

- Methanolic extracts of *Phyla nodiflora* at a dose of 200mg/kg, 400mg/kg & 800mg/kg of the crude extract are administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses.

4.6.4.2. Hole Board Test:

- Methanolic extracts of *Phyla nodiflora* at a dose of 200mg/kg, 400mg/kg & 800mg/kg of the crude extract are administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses.

4.6.5. Methods for CNS Activity Test:

To determine CNS effect of the plant extract two different methods are used with different groups of testing animals. These methods are-

- Open Field Test.

- Hole Board Test.
- After the extraction of the plant, each group is treated with the extract in order to determine some specific parameters according to the experimental protocol.

4.6.5.1. Open Field Test:

In this experiment, the method according to Gupta, 1971 was employed. An open field, a test paradigm which is highly standardized to evaluate locomotor activity (Kelley, 1993). The animals were divided into negative control, positive control and test groups containing twelve mice in each group. Negative control group received vehicle (5% CMC solution) at a dose of 10 mg/kg body weight orally. The test groups received extracts of *Phyla nodiflora* at the doses of 200mg/kg, 400 mg/kg & 800mg/kg body weight orally. The floor of an open field of half square meter was divided into a series of squares, each alternatively colored black and white. It has 49 squares. The number of Peripheral locomotion (movement of mice on surrounding 40 squares other than central 9 squares), number of Central locomotion (movement of mice on central 9 squares), number of Leaning (standing of mice with the help of wall) and number of Rearing (standing of mice without any help) number of Grooming (face rubbing or itching), and number of defecation was recorded for a period of two minutes. The observation was conducted at 0, 30, 60, 90 and 120 minutes after oral administration of test drugs and was compared with control animal.



Figure 4.5: Open Field Test

4.6.5.2. Hole Board Test:

The hole board represents a combination of a hole board, originally designed to investigate explorative motivation in rodents (Lister, 1990) and later on modified to evaluate cognitive functions (Ohl and Fuchs, 1999; Ohl et al., 1998) 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. This experiment was carried out by the following method of Boisser and Simon, (1964). The animals were divided into negative control and test groups containing six mice in each group. Negative control group received vehicle (5% CMC solution) at a dose of 10 mg/kg body weight orally. The test groups received extracts *Phyla Nodiflora* at the doses of 200mg/kg, 400 mg/kg & 800mg/kg body weight orally. Each of the animal 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 and post 30 minutes intervals and were compared with the control animals



Figure 4.6 : Hole Board Test

4.7. Toxicity Test

4.7.1 Materials for Toxicity Test

- Analytical Balance,
- Feeding needle: 1 c.c.
- Insulin syringes 100 units disposable
- 5 ml syringe disposable

- Dissecting box
- Dissecting pad
- Pin
- Beaker 1 litre
- Petri dish for washing
- Epindrop tube
- 250 ml food grade plastic pot
- Gloves
- Mask

4.7.2 Chemical Agents Used Toxicity Test

- 5% CMC (Vehicle) 10ml/kg as negative control,
- Saline water (0.9%)
- Formalin (5%)
- EDTA
- Heparin
- Choloform

4.7.3 Doses Used for Toxicological Activity of the Extract:

4.7.3.1 Acute Toxicity Test:

Methanolic extracts of *Phyla Nodiflora* at a dose of 2000mg/kg 4000mg/kg and 8000mg/kg were administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses.

4.7.3.2. Chronic Toxicity Test:

Methanolic extracts of *Phyla Nodiflora* at a dose of 200mg/kg, 400 mg/kg & 800mg/kg are administered orally. 5% CMC is used as a vehicle with plant methanolic extract for preparing different doses.

4.7.4 Methods for Toxicity Test:

4.7.4.1. Acute Toxicity Test

The acute toxicity of in Swiss albino mice was studied as reported method. Each extract were given to three groups (n = 6) of mice at 2000mg/kg ,4000mg/kg,8000 mg/kg body weight, orally. The treated animals were kept under observation for 3 days, for mortality and general behaviour. (Paul, et.al. 2012).

4.7.4.2. Chronic Toxicity Test

The adult Swiss albino mice were divided into five groups containing 12 animals per group. The two groups(male & female) received 5% CMC (Vehicle) 10ml/kg and the other three groups received the three doses of extracts like 200 mg/kg, 400 mg/kg, 800 mg/kg according to body weight orally, respectively daily for 90 consecutive days. Food and water intake of animals were observed during this period. Body weight was taken for every 3 days. Twenty four hours after the last dose (i.e., at the 91th day), the mice were fainted by using chloroform and collected blood using 5 ml disposable syringe from cardiac puncture and reserved it in both heparinized and non- heparinized Epindrop tube. Then also collected other organ like Brain, Liver, Kidneys, Heart, Lung, and Stomach and reserved it food grade plastic pot having 5% formalin. Then this blood and liver was used for the study of Hematology test, Protein Test and Liver biochemical parameters Test (Paul, et.al. 2012).

4.7.5. Hematological parameters

Collected blood was used for the estimation of hemoglobin (Hb) content; red blood cell count (RBC) and white blood cell count (WBC).(Estimations are carried out by using the **Sysmex XT 2000i** Hematology Analyzer ,CARE Specialized Hospital and Research Centre Ltd, Dhaka, Bangladesh)

4.7.6. Serum biochemical parameters

Collected blood was used for the estimation of serum biochemical parameters like serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP) contents by using commercially available reagent kits(CARE Specialized Hospital and Research Centre Ltd, Dhaka, Bangladesh)



Figure 4.7 : Mice Organ

4.7.7. Histopathological studies

After sacrifice the organs like heart, lung, liver, kidney and pancreas of animals from each group were subjected for histopathological examinations. After fixing the tissues in 10% formaldehyde the tissues were dehydrated and paraffin blocks were made. Then sectioning was done at about 5-7 μ . Routine histopathology was performed by using the Haemotoxylin stain (Paul, et.al., 2012).

4.7.8. Statistical Analysis

Data obtained from pharmacological experiments are expressed as mean \pm SEM. Difference between the control and the treatments in these experiments were tested for significance using

one-way analysis of variance (ANOVA), followed by Dunnet's t-test for multiple comparisons using SPSS -16 software.



Figure 4.8 : Collecting the blood and organ of mice

Chapter 5

Result and Discussion

5. Result and Discussion

5.1. *Phyla Nodiflora*:

Crude extract of *Phyla Nodiflora* were subjected to evaluate the gastric motility effects of the plant on different experimental models. A series of *in vivo* pharmacological experiments were carried out to determine laxative effect of the plant.

5.1.1. Charcoal meal GI transit test:

For the determination of laxative effect, charcoal meal GI transit test was done upon the administration of the crude extract of *Phyla Nodiflora*. Table-5.1 and figure-5.1 show the laxative effect by charcoal meal GI transit test of the crude extract of *Phyla nodiflora* on normal and atropine induced test mice. For the determination of GI transit rate, the length of small intestine and the distance between the pylorus region and front of the charcoal meal was measured to obtain the charcoal transport ratio or percentage. The test was carried out to find out the effects of extract on the transit of the gastrointestinal tract. Comparative evaluation of the extract with the reference motility drug, bisacodyl, and Negative control group showed that the extract significantly increase gastrointestinal motility in mice. A total 8 doses, e.g. 200 mg/kg, 400 mg/kg & 800mg/kg , 400mg/kg + atropine, and 800mg/kg + atropine of crude extract of *Phyla nodiflora* were used for the gastrointestinal transit test.

Pharmacological Study Of *Phyla Nodiflora*

Table 5.1: Effects of crude extract of *Phyla nodiflora* on the Gastrointestinal Transit Test:

Group No.	Treatment	Dose (ml/kg or mg/kg)	Mean of % length of small intestine
1	5% CMC (p.o)	10	34.2 ± 1.07***
2	Bisacodyl (p.o)	5	80.8 ± 0.86***
3	Dose-1, P.Nodiflora (p.o)	200	52.6 ± 1.03***
4	Dose-2, P.Nodiflora (p.o)	400	71.6 ± 1.25***
5	Dose-3, P.Nodiflora (p.o)	800	80 ± 1.41***
6	Bisacodyl (p.o)+Atropine (i.p)	5 + 10	73 ± 1.22***
7	Dose-2 (p.o)+ +Atropine (i.p)	400+10	64.2 ± 1.11***
8	Dose-3 (p.o)+ +Atropine (i.p)	800+10	73.4 ± 1.57***

Dose-1=200mg/kg (P.Nodiflora)

Dose-2= 400 mg/kg (P.Nodiflora)

Dose-3= 800 mg/kg (P.Nodiflora)

P.Nodiflora = *Phyla nodiflora*

p.o=Per oral; i.p= Intraperiotinal

All the test group of crude extract except group-3 showed significant increased transit ($p < 0.001$, $p < 0.01$ and $p < 0.05$) of GI motility test at a dose of 200 mg/kg, 400mg/kg and 800 mg/kg. For each doses (Dose-1, 2, 3) two groups of animals were tested. One group was given only dose and another group was pretreated with atropine (i.p) before administration of doses. Among all the

test groups of crude extract, dose-3 (group-5) showed highest percentage of the intestinal length traversed by the charcoal (80 ± 1.41 , $p < 0.001$). The reference drug bisacodyl showed the percentage of length 80.8 ± 0.86 ($p < 0.001$) at a dose of 5mg/kg.

In case of the test group of the animals pre-treated with atropine also showed significant increase of percentage of length travelled by charcoal. Crude extract of group 8 showed most prominent percentage of the intestinal length traversed by the charcoal, that is (73.4 ± 1.57 , $p < 0.001$) and the reference drug showed the percentage of length (73 ± 1.22 , $p < 0.001$).

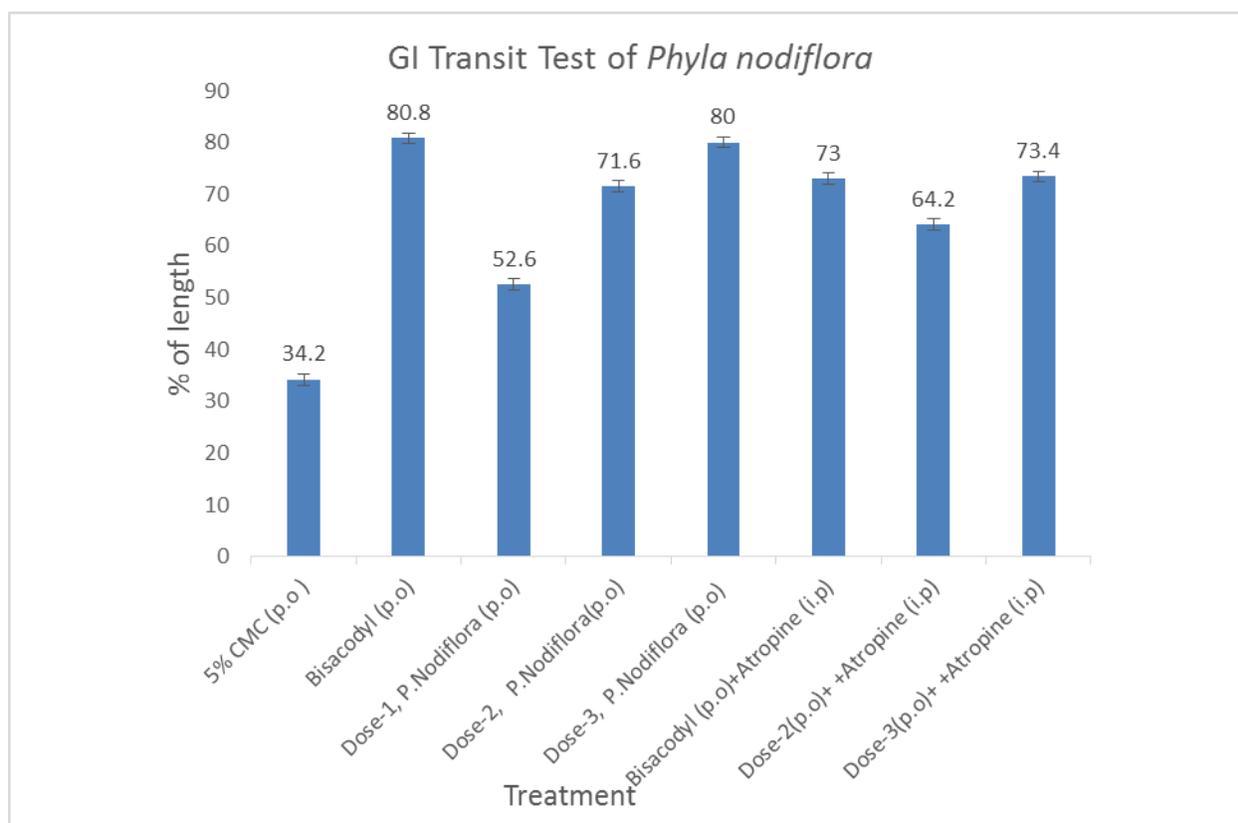


Figure 5.1: Bar diagram showing the dose-dependent effect of crude extract on the travel of charcoal meal through small intestine of mice, in the absence and presence of atropine. Each bar shown represents mean of 5 animals per group.

5.2 CNS Activity Test of Methanolic Extract of *Phyla Nodiflora*

5.2.1. Open Field Test:

Pharmacological Study Of *Phyla Nodiflora*

CNS of the methanolic extract of plant *Phyla nodiflora* studied in different doses (200, 400 and 800mg/Kg body weight) of the crude extract, using diazepam as a positive control. The extract produced effects at doses of 200, 400 and 800 mg/kg body weight respectively (Table 5.2, 5.3 and 5.4 and Fig. 5.2, 5.3 and 5.4). The result was found to be statistically significant. The experimental findings that are noted are below-

5.2.1.1. Negative Control Group (5% CMC, 10 ml/kg)

This group of animals only received vehicle (5% CMC) 10 ml/kg orally.

- ✓ The observed total number of rearing count is followed with a mean value of at 0 min , 30 min, 60 min, 90 min and 120 min was 0 during 2 minutes observation.
- ✓ The observed total number of grooming count is followed with a mean value of at 0 min $11 \pm 1.53^{***}$ (Mean \pm SEM) at 30 min, 60 min, 90 min, and 120 min was 0 during 2 minutes observation.
- ✓ The observed total number of defecation count is followed with a mean value of at 0 min $0.5 \pm 0.22^{**}$ at 30 min 0.33 ± 0.21 at 60 min 0.17 ± 0.16 at 90 min 0.5 ± 0.22 and at 120 min 0.5 ± 0.22 (Mean \pm SEM) during 2 minutes observation.

5.2.1.2. Positive Control Group (Diazepam, 1mg/kg)

This group of mice receives the standard drug diazepam of 1mg/kg orally.

- ✓ The observed total number of rearing count is followed with a mean value of at 0 min , 30 min, 60 min, 90 min and 120 min was 0 during 2 minutes observation.
- ✓ The observed total number of grooming count is followed with a mean value of at 0 min $15.53 \pm 0.88^{***}$ (Mean \pm SEM) at 30 min 0, at 60 min 0, at 90 min 0 and at 120 min 0 during 2 minutes observation.
- ✓ The observed total number of defecation count is followed with a mean value of at 0 min $0.5 \pm 0.22^{**}$ at 30 min 0.5 ± 0.22 , at 60 min 0.33 ± 0.21 , at 90 min 0.33 ± 0.21 and at 120 min 0.17 ± 0.16 (Mean \pm SEM) during 2 minutes observation

5.2.1.3. 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 rearing count is followed with a mean value of at 0 min , 30 min, 60 min, 90 min and 120 min was 0 during 2 minutes observation.
- ✓ The observed total number of grooming count is followed with a mean value of at 0 min 11.17±1.08*** at 30 min 6.17±0.65*** at 60 min, at 90 min, and at 120 min 0(Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of defecation count is followed with a mean value of at 0 min 1.0±0.37* at 30 min 1.17±0.31* , at 60 min 0.83±0.31*, at 90 min 0.33±0.21 and at 120 min 0.67±0.21* (Mean ±SEM) during 2 minutes observation.

5.2.1.4. Test Group-2 (Plant Extract, 400mg/kg)

These groups of mice receive the plant extract of 400 mg/kg orally.

- ✓ The observed total number of rearing count is followed with a mean value of at 0 min , 30 min, 60 min, 90 min and 120 min was 0 during 2 minutes observation.
- ✓ The observed total number of grooming count is followed with a mean value of at 0 min 13.5±1.23*** at 30 min 6.0±0.58*** at 60 min, 90 min, and at 120 min 0 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of defecation count is followed with a mean value of at 0 min 1.17±0.31* at 30 min 1.0±0.2*, at 60 min 0.5±0.22*, at 90 min 0.67±0.21* and at 120 min 0.5±0.34 (Mean ±SEM) during 2 minutes observation.

5.2.1.5. Test Group-3 (Plant Extract, 800mg/kg)

These groups of mice receive the plant extract of 800 mg/kg orally.

- ✓ The observed total number of rearing count is followed with a mean value of at 0 min , 30 min, 60 min, 90 min and 120 min was 0 during 2 minutes observation.

Pharmacological Study Of *Phyla Nodiflora*

- ✓ The observed total number of grooming count is followed with a mean value of at 0 min 20.83±1.24*** at 30 min 14.83±1.01*** at 60 min, 90 min, and at 120 min 0 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of defecation count is followed with a mean value of at 0 min 2.17±0.31** at 30 min 1.33±0.21**, at 60 min 1.17±0.31*, at 90 min 0.83±0.31* and at 120 min 0.83±0.4 (Mean ±SEM) during 2 minutes observation.

Table 5.2: CNS Activity of plant extract of *Phyla Nodiflora* by Open Field Test (Peripheral Locomotion) in Mice.

Groups	Dose	No. of Peripheral Locomotion				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	109.33±3.3***	107.67±2.7***	76.83±3.58***	88.83±1.89***	88.17±3.39***
Crude extract of <i>Phyla nodiflora</i>	200mg/kg	114.17±1.66***	78.5±1.93***	64.17±1.75***	47.67±1.56***	38.33±0.88***
Crude extract of <i>Phyla nodiflora</i>	400mg/kg	98.67±2.26***	76.67±1.33***	59.17±1.17***	38.33±0.88***	31.5±1.52***
Crude extract of <i>Phyla nodiflora</i>	800mg/kg	87.17±2.01***	58.5±1.36***	42.83±2.4***	26.17±1.16***	26±2.07***
Positive control, Diazepam	1mg/kg	121.83±1.1***	69.33±1.12***	53.0±1.81***	35.67±1.17***	27.83±1.72***

Each value is the mean ± SEM for 6 mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

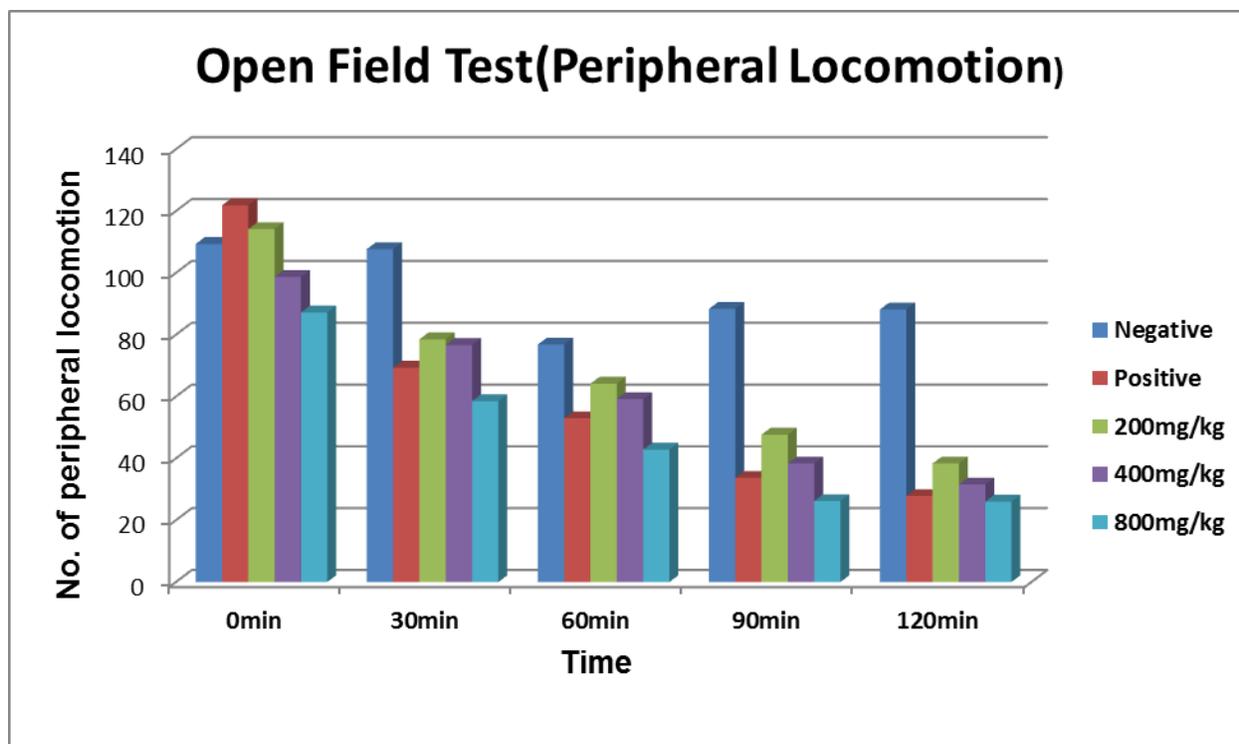


Figure 5.2: Graphical Presentation of CNS Activity of plant extract of *Phyla nodiflora* by Open Field Test (Peripheral Locomotion) in Mice.

Table 5.3: CNS Activity of plant extract of *Phyla Nodiflora* by Open Field Test (Central Locomotion) in Mice.

Groups	Dose	No. of Central Locomotion				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	20.0±1.32***	11.33±1.26***	14.5±1.98**	12.33±1.52** *	13.5±1.43***
Crude extract of <i>Phyla nodiflora</i>	200mg/kg	15±0.78***	8.17±0.48***	4.5±0.42***	3.83±0.31***	2.67±0.21***
Crude extract of <i>Phyla nodiflora</i>	400mg/kg	15±0.37***	10.17±0.6***	6.33±0.42***	3.83±0.31***	2.33±0.21***
Crude extract of <i>Phyla nodiflora</i>	800mg/kg	8.67±0.67***	4.17±0.31***	3.17±0.31***	2.5±0.22***	2.33±0.21***
Positive control, Diazepam	1mg/kg	20.67±1.05**	9.5±0.76***	6.17±0.6***	4.17±0.6**	3.33±0.42**

Each value is the mean ± SEM for 6 mice, * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test

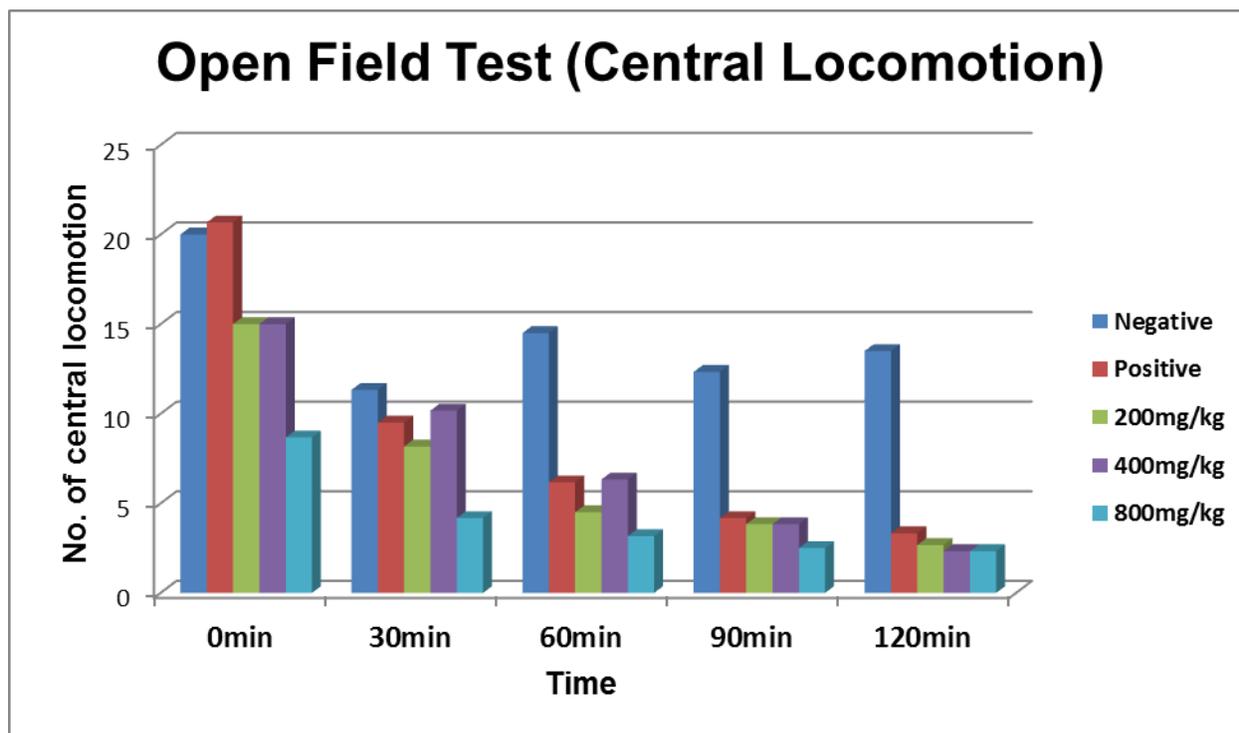


Figure 5.3: Graphical Presentation of CNS Activity of plant extract of *Phyla nodiflora* by Open Field Test (Central Locomotion) in Mice.

Pharmacological Study Of *Phyla Nodiflora*

Table 5.4: CNS Activity of plant extract of *Phyla Nodiflora* by Open Field Test (Leaning) in Mice.

Groups	Dose	No. of Leaning				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	16.67±1.54***	13.5±0.76***	9±0.73***	10.0±0.58***	12.83±1.4***
Crude extract of <i>Phyla nodiflora</i>	200mg/kg	16.83±0.91***	11.33±0.49***	9.17±0.48***	7.0±0.37***	4.33±0.33***
Crude extract of <i>Phyla nodiflora</i>	400mg/kg	14.83±0.7***	9.5±0.56***	7±0.37***	4.83±0.31***	3.5±0.22***
Crude extract of <i>Phyla nodiflora</i>	800mg/kg	12.33±0.56***	6.5±0.43***	4.0±0.26***	2.5±0.22***	2.17±0.31**
Positive control, Diazepam	1mg/kg	22.17±1.08***	8.83±0.31***	6.17±0.31***	4.33±0.33***	3.17±0.48**

Each value is the mean ± SEM for 6 mice, * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

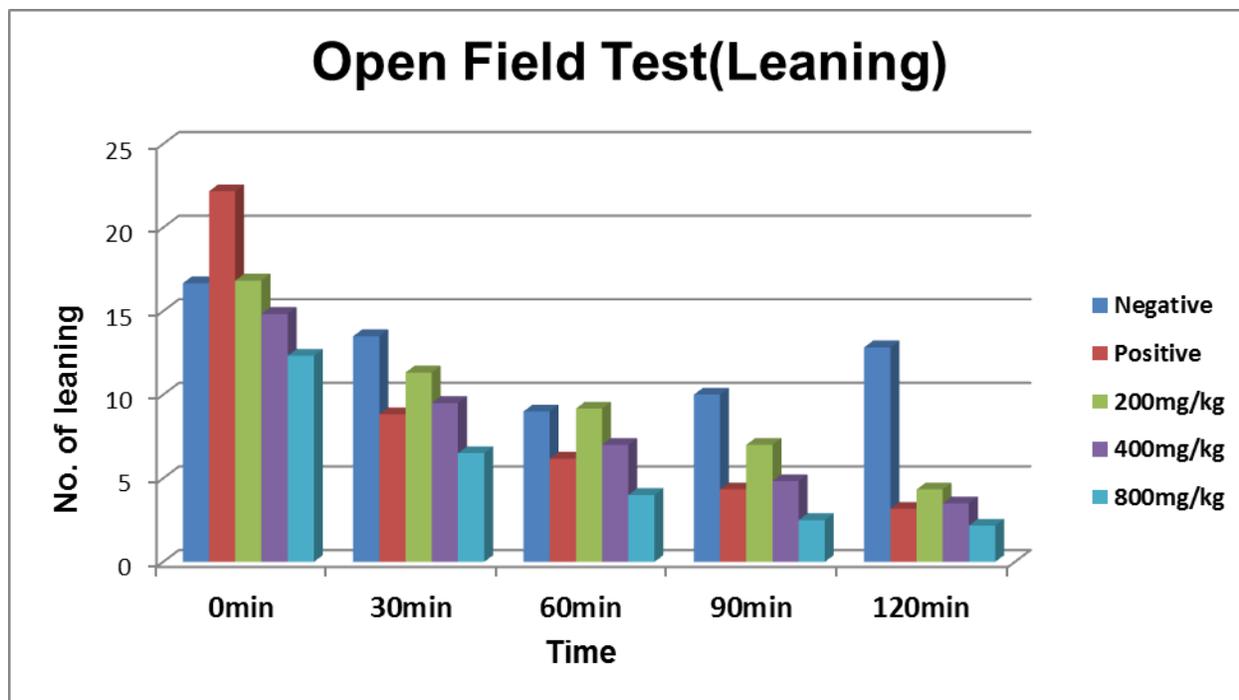


Figure 5.4: Graphical Presentation of CNS Activity of plant extract of *Phyla Nodiflora* by Open Field Test (Leaning) in Mice.

5.2.2. Hole Board Test:

CNS of the methanolic extract of the bark part of the plant *Phyla Nodiflora* studied in different doses (200, 400 and 800mg/Kg body weight) of the crude extract, using diazepam as a positive control. The extract produced effects at doses of 200, 400 and 800 mg/kg body weight respectively (Table 5.5, and Fig. 5.5). The result was found to be statistically significant. The experimental findings that are noted are below-

Table 5.5: CNS Activity of plant extract of *Phyla Nodiflora* by Hole Board Test in Mice.

Groups	Treatment	Dose	No. of Head Poking	No. of Head Dipping
Negative control	5% CMC	10ml/kg	68.5±1.48***	25.17±1.01***
Group-1	Crude extract of <i>Phyla nodiflora</i>	200mg/kg	44.58±1.09***	17.75±0.91***
Group-2	Crude extract of <i>Phyla nodiflora</i>	400mg/kg	34.5±1.08***	15.25±0.66***
Group-3	Crude extract of <i>Phyla nodiflora</i>	800mg/kg	34.45±1.44***	12.09±0.77***
Positive control	Diazepam	1mg/kg	29.83±1.01***	15.67±0.67***

Each value is the mean ± SEM for 6 mice, * P < 0.05; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

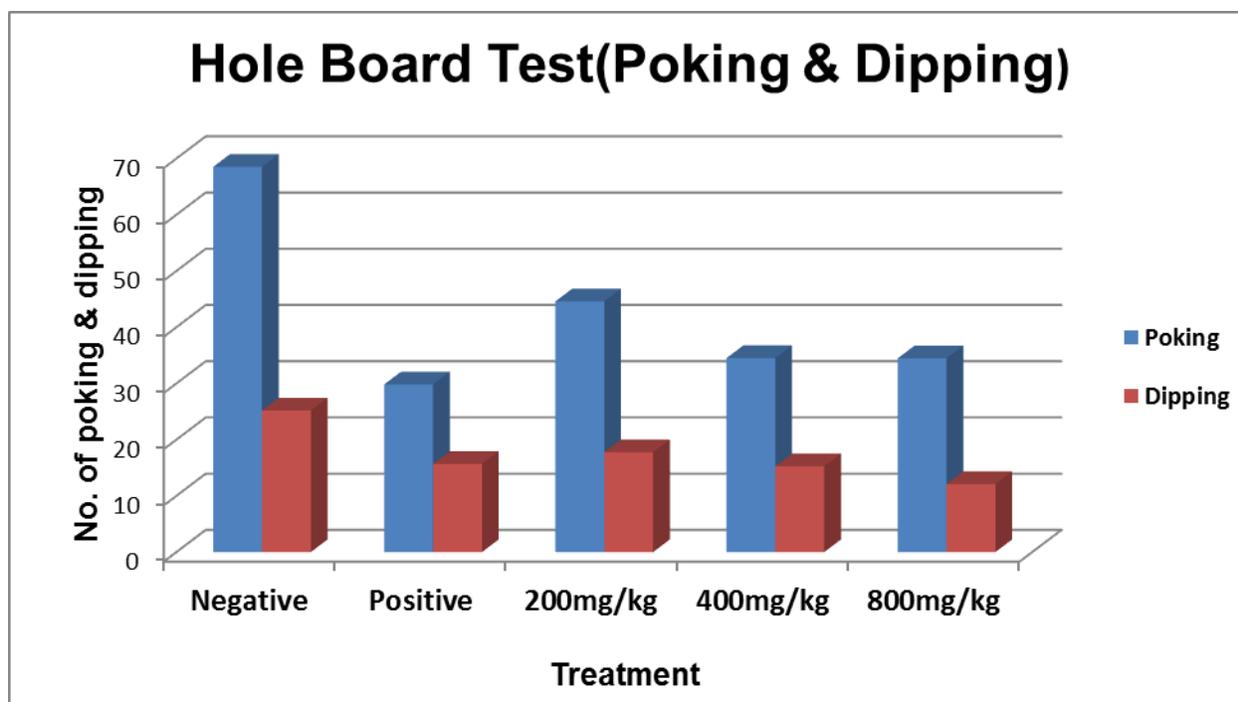


Figure 5.5: Graphical Presentation of CNS Activity of plant extract of *Phyla Nodiflora* by Hole Board Test in Mice.

5.3. Acute and Chronic Toxicity Test:

5.3.1. Acute toxicity: For 3 days observation no death was observed till the end of the study.

5.3.2. Chronic Toxicity Test:

5.3.2.1. CBC (Count Blood Cell) Test, Biochemical Test:

Drug dose 400 and 800 mg/kg (CBC & Biochemical Test):

In the chronic study of methanolic extract of *Phyla nodiflora* at a dose (400,800 mg/kg) to the mice, significant difference were not found in the erythrocyte and leucocytes values of both the treated and control mice. In which case, the administration of *Phyla nodiflora* methanolic extract for a period of 90 days cannot induce significant anaemia. Though minor irregularities were observed mainly in the RBC, WBC, Platelet, SGPT, SGOT and ALP (hepatic enzymatic test) this could be as a result of the mice response to foreign bodies associated with the chronic toxicity during the experiment. The toxicity assay did not result any abnormality and mortality of

the tested mice for the period of 90 days monitored. With this result where no adverse effect was seen in the administration of *Phyla nodiflora*.

Table 5.6 : Effect of methanolic extract of *Phyla nodiflora* on body weight in mice

Treatment Groups	Gender	Initial body weight	Final body weight	No. of death
Normal Control	Female	20.11±1.08***	27.14±1.32***	0
Normal Control	Male	22.24±1.54***	30.19±1.93***	0
<i>Phyla nodiflora</i> 200mg/kg	Female	29.1±1.58***	35.7±1.10***	0
<i>Phyla nodiflora</i> 800mg/kg	Male	29.69±1.65***	40.38±1.87***	0

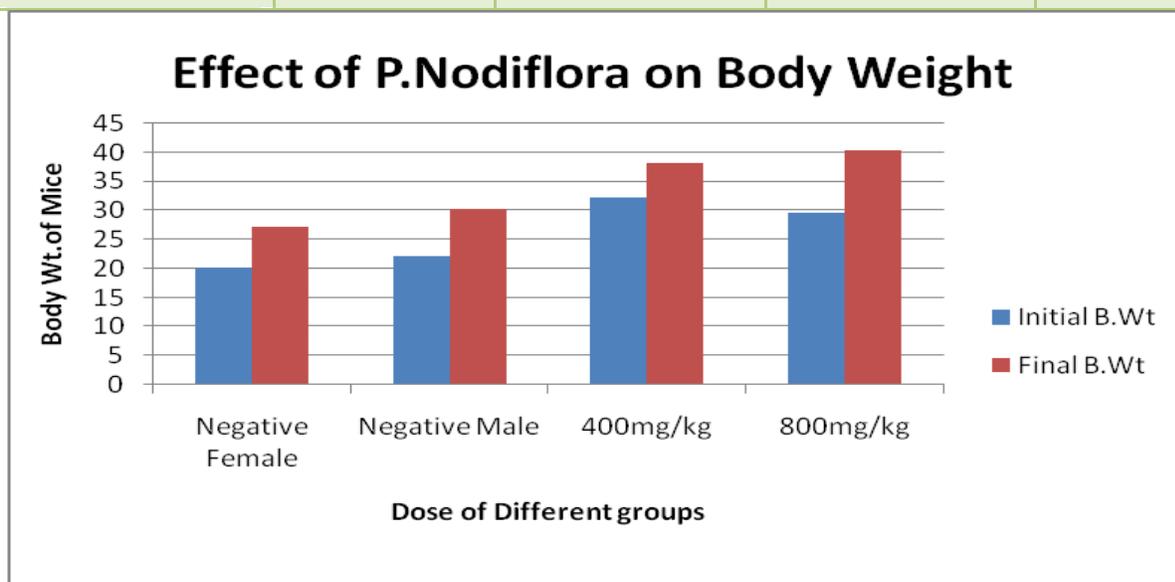


Figure 5.6: Graphical Presentation of Effect of methanolic extract of *Phyla nodiflora* on body weight in mice

Table 5.7: Effect of *Phyla nodiflora* on the count of WBC (White Blood Cell)

Treatment Group	Total WBC 10 ³ /mm ³ (n)	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Negative Control group(Female)	3.9	19.72	76.07	2.9	0.68	0.75
Negative Control group(Male)	5.43	18.32	76.18	4.15	0.67	0.68
<i>Phyla nodiflora</i> (400mg/kg, Female)	5.5	30.5	59.2	8.63	1.67	0.78
<i>Phyla nodiflora</i> (800mg/kg, Male)	8.042	29	65.5	4.25	1.217	0.1

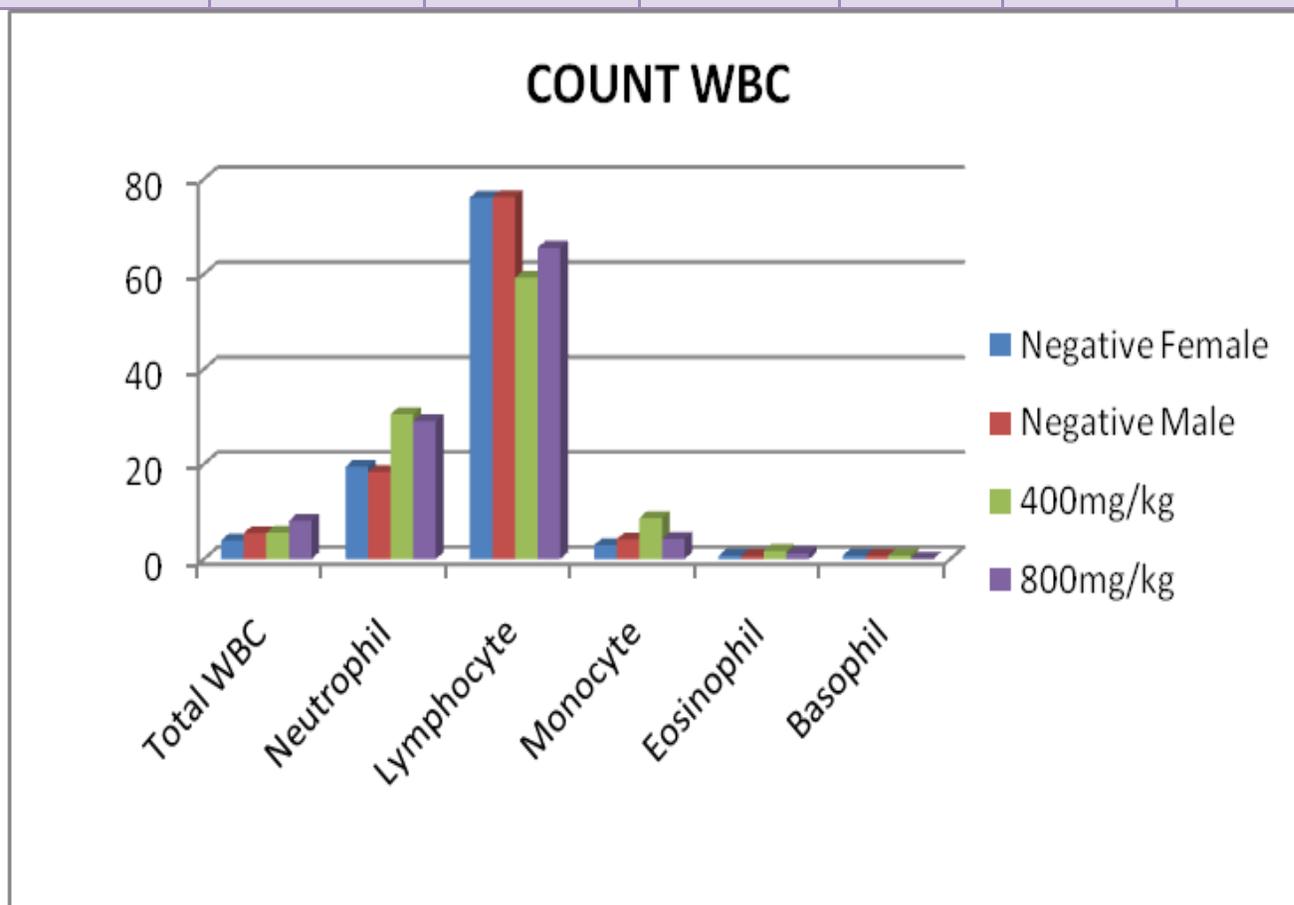


Figure 5.7 : Effect of *Phyla nodiflora* on the Different count of WBC (White Blood Cell)

Table 5.8 : Effect of *Phyla nodiflora* on the count of RBC (Red Blood Cell)

Treatment Group	Total RBC10 ⁶ /mm ³ (n)	Haemo globin	HCT	MCV	MCH	MCHC	RDW
Negative Control group(Female)	8.33	14.5	55.5	64.25	16.62	25.9	21.58
Negative Control group(Male)	8.79	13.55	50.3	56.7	15.28	26.93	22.28
<i>Phyla nodiflora</i> (400mg/kg, Female)	9.4	13.8	52.5	55.7	14.6	26.3	25.6
<i>Phyla nodiflora</i> (800mg/kg, Male)	9.217	14.07	52.73	57.33	15.23	26.58	24.85

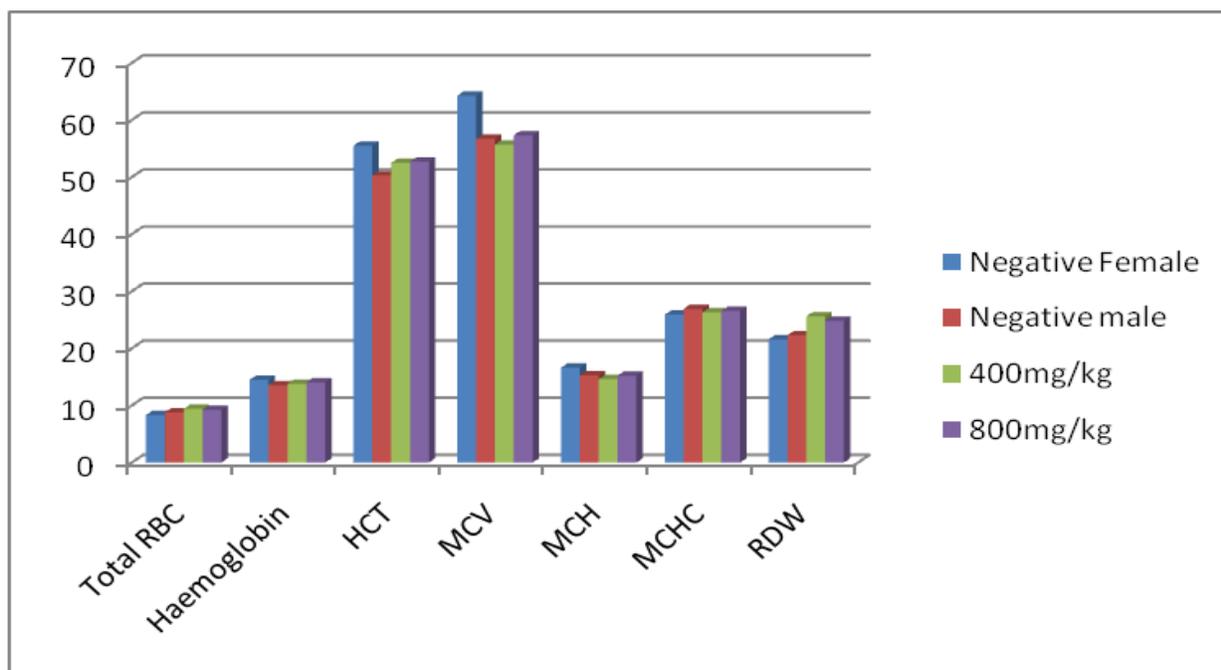


Figure 5.8: Effect of *Phyla nodiflora* on the count of RBC (Red Blood Cell)

Table 5.9: Effect of *Phyla nodiflora* on Platelet and MPV count on the CBC (Count Blood Cell) Test

Treatment Group	Platelet	MPV
Negative Control group(Female)	848	8.3
Negative Control group(Male)	1211	7.75
<i>Phyla nodiflora</i> (400mg/kg, Female)	1029	8.9
<i>Phyla nodiflora</i> (800mg/kg, Male)	1111	8.583

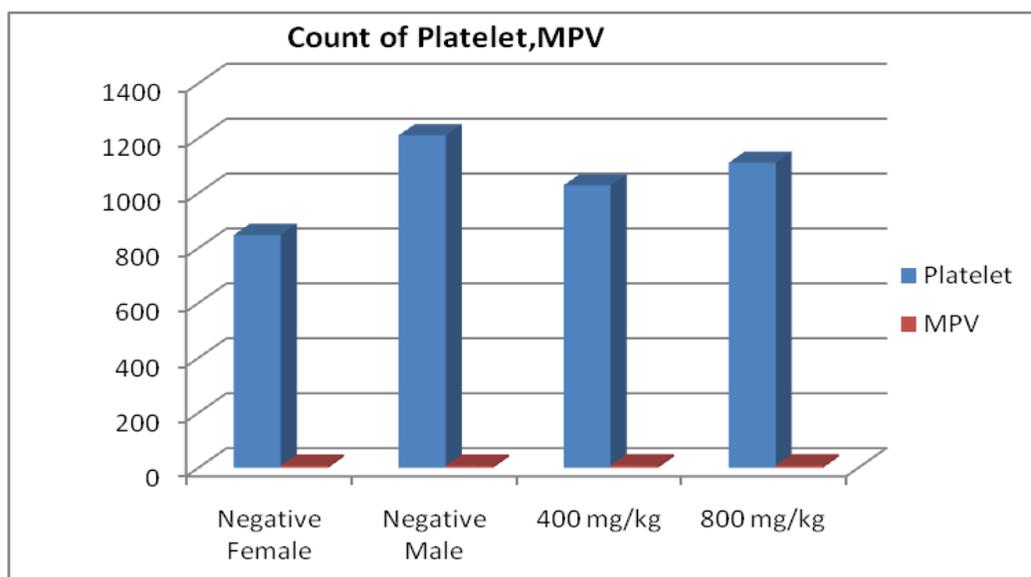


Figure 5.9: Effect of *Phyla nodiflora* on Platelet on the CBC (Count Blood Cell) Test

Table 5.10: Effect of *Phyla nodiflora* on the Liver Function Test

Treatment Group	SGPT (IU/dl)	SGOT (IU/dl)	SALP (IU/dl)
Negative Control group(Female)	55.83	33.33	102.17
Negative Control group(Male)	49.17	31.83	341.5
<i>Phyla nodiflora</i> (400mg/kg, Female)	16.7	43.7	88.3
<i>Phyla nodiflora</i> (800mg/kg, Male)	30.8	43.4	87.8

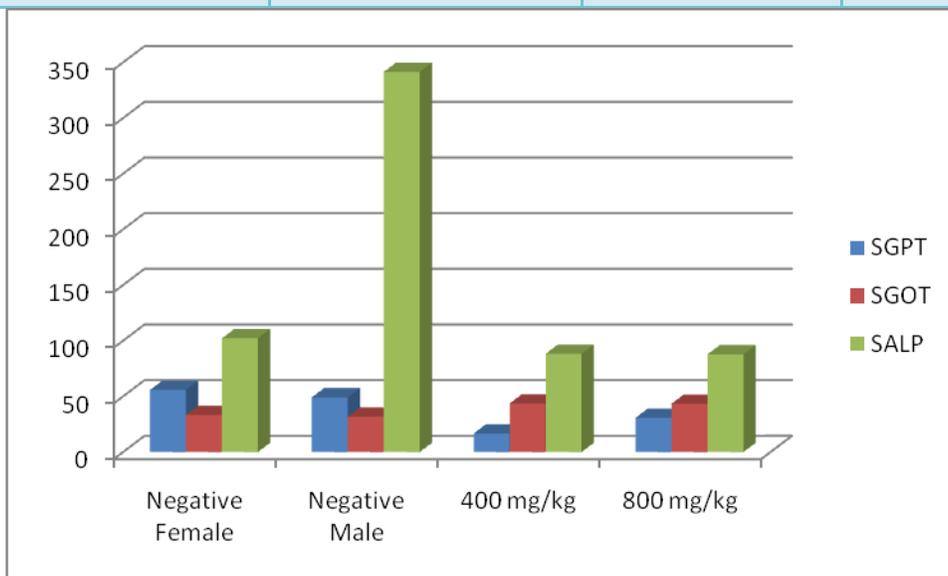


Figure 5.10: Effect of *Phyla nodiflora* on the Liver Function Test

Chapter 6

Conclusion

6. Conclusion

Traditional medicines are mostly utilized by means of the natural products isolated from natural resources such as plant extracts. Pharmacological studies always reveal the potential medicinal properties of plants of our surroundings. Ethnobotanical data on the traditional uses of plants encourage the isolation of secondary metabolites leading to new lead compounds. With the increasing demands of inventing new drugs the pharmacological assay of natural plant resources play an unparalleled role in traditional drug discovery. Day by day the study of traditional medicinal plants is increasing in significant rate with the view to invention and establishment of new therapy line.

As a part of our project aimed at the pharmacological evaluation of a medicinal plant, I studied the Laxative activities, Central Nervous System activities, Acute and Chronic toxicity of methanolic extract of *Phyla nodiflora*.

This study shows that the prokinetic and laxative activities of extracts of *Phyla nodiflora* in mice are partially mediated through muscarinic receptors. Thus, this study provides sound mechanistic basis for the medicinal use of *Phyla nodiflora* in gut disorders, such as indigestion and constipation.

The plant extract was also assessed on the central nervous system using a number of neuropharmacological experimental models in mice. The crude extract of *Phyla nodiflora*. (200mg/kg, 400mg/kg & 800mg/kg) dose dependently reduces the number of peripheral locomotion, central locomotion and leaning in the open field test. The reduction is significant (***) $p < 0.001$) when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Diazepam 1mg/kg. The crude extract of *Phyla nodiflora*, (200mg/kg, 400mg/kg & 800mg/kg) also dose dependently reduces the number of head dipping and head poking in the hole board test. The reduction is significant (***) $p < 0.001$) when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Diazepam 1mg/kg. The reference drug is found slightly potent than the extract.

The aim of the study was also to investigate the possible toxicity of the plant *Phyla nodiflora* and especially to establish the safety of the methanolic extract of this plant by focusing on its acute and chronic toxicity in mice. For finding chronic toxicity several tests are done such as CBC (Cell Blood count) test, Hepatic enzyme. CBC test and hepatic enzyme test are done by

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hematological machine. The results of several widely accepted protocols would suggest that there were positive modulations in all the parameters of study in the *Phyla nodiflora* extract, in which significant difference were not found in RBC and different count of RBC, WBC & different count of WBC, hepatic enzyme (SGPT, SGOT & SALP) values of treated mice. The result shows that the toxic effect of methanolic extract of *Phyla nodiflora* is safe in mice that is no significant change with dose when compare with negative control.

From the present investigation, it can be concluded that the methanolic extract of *Phyla nodiflora* exhibited Laxative activity, Depressant activity, and shows toxicity safety in acute and chronic toxicity studies in mice.

Chapter 7

Reference

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