Study of Pharmacological Activities of Methanolic Extract of *Aglaonema hookerianum Leaf*

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

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Declaration by the Candidate

I, **Syeed Ashfaq Ahmed (ID:2014-1-70-073)**,hereby declare that the dissertation entitled **"Study of Pharmacological Activities of Methanolic Extract of *Aglaonema hookerianum* Leaf "** submitted by me to the Department of Pharmacy, East West University, in the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, work carried out by me during the year 2017 of my research in the Department of Pharmacy, East West University, under the supervision and guidance of, Dr. Shamsun Nahar Khan, Associate Professor, Department of Pharmacy, East West University. The thesis paper has not formed the basis for the award of any other degree/diploma/fellowship or other similar title to any candidate of any university.

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Certificate by the Supervisor

This is to certify that, the research work on **"Study of Pharmacological Activities of Methanolic Extract of *Aglaonema hookerianum* Leaf "** submitted to Department of Pharmacy, East West University, Jahurul Islam city, Aftabnagar, Dhaka-1212, in partial fulfillment of the requirements for the degree of Masters of Pharmacy (B. Pharm) was carried out by **Syeed Ashfaq Ahmed (ID: 2014-1-70-073)** 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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This is to certify that the thesis entitled **"Study of Pharmacological Activities of Methanolic Extract of *Aglaonema hookerianum* Leaf "** submitted to the Department of Pharmacy, East West University for the partial fulfillment of the requirement for the award of the degree Bachelor of Pharmacy, was carried out by **Syeed Ashfaq Ahmed (ID: 2014-1-70-073)**,during the period 2017 of her research in the Department of Pharmacy, East West University.

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Acknowledgement

At first, I would like to thanks the Almighty **ALLAH** the most gracious and merciful for enabling me to successfully completing my research work soundly and orderly.

I would like to express my deepest gratitude to my research supervisor, **Dr. Shamsun Nahar Khan**, Associate Professor and Chairperson, Department of Pharmacy, East West University, for his suggestion, careful guidance, sincere help, constructive criticism and valuable time without which I would not have been able to complete this work. He had been very enthusiastic and supportive in my research.

I put forward my sincere regards and profound gratitude to Chairperson, **Dr. Chowdhury Faiz Hossain**, Professor, Department of Pharmacy, East West University, for his inspiration in my study. He also paid attention for the purpose of my research work and extending the facilities to work.

I express my sincere thankfulness to my parents and my sister for their support and guidance all through my life, including that for my research project.

I am thankful to the laboratory instructors Mr. Sujit Kumar, Razia Sultana Shipra Biswas and Ruchira Chakraborty, for their kind support during the laboratory work.

I want to give special thanks to, Mohammad Ali for his kind help. I also would like to thanks Sabrina Islam, Shirajum Monira, Md Abu Hanif, Romen Royhan, Abid Hasan, Shaikh Rasel, Farjana Sarwar, Amreen Ahmed, Samiha Tammana, Sohanur Rahman for their support and guidance throughout this research project. My hearfelt gratitude goes to my all friends, who gave me support for my research work and for their extended cooperation for my study.

I also want to remember all of the stuffs of Pharmacy Department with a thankful heart who helped me a lot to complete this research work successfully. During the course of this research work, a lot of experiences I have received which is of inestimable value for my life.

Abstract

Purpose: The research work was carried out to determine the pharmacological activities of methanolic extract of *Aglaonema hookerianum*

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. 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 *Aglaonema hookerianum.* (200mg/kg, 400mg/kg & 800mg/kg) dose dependently reduces the number of peripheral locomotion, central locomotion and leaning in the open field test but increases the number of grooming. The reduction is significant (***$p<0.001$) when it is compared to the standard drug.

In the methanolic extract of Aglaonema hookerianum(Leaf) it showed highest 41.75% thrombolytic activity and moderate activity showed by the above experiment compared with the streptokinase and *Aglaonema hookerianum(* Bark).

In total phenolic content assay of methanolic extact of *Aglaonema hookerianum* have the phenolic content of (122.6 mgSAE/g). In DPPH radical activity of methanolic extract of *Aglaonema hookerianum* is good (IC_{50} 11.0(μg/ml). In reducing power assay higher absorbance of the reaction mixture indicates higher reductive potential. In this assay, *Aglaonema hookerianum* shows as the concentration increases the absorbance is also increase

.

The aim of the study was also to investigate the possible toxicity of the plant *Aglaonema hookerium* 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 test and histopathological Studies.

All data were analyzed by using SPSS analytical method.

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

Keywords: *Aglaonema hookerianum*, Laxative effect, Neuropharmacological effect , Antioxidant effect, Thrombolytic activity and Toxicity test.

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Chapter 01

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 anti-ashmatics (86.79 %), anti-rheumatics (62%), diuretics (60.22%), anti-inflammation (29.62 %), anticancer (9.75 %), anti-diabetics (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 treatment etc. (Ghani, 2003).

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. (*Farnsworth, N.R. and Soejarto, D.D., 1991*)

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. (*Farnsworth, N.R. and Soejarto, D.D., 1991*).

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 posses medicinal 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, andhepatotoxicity, 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: p*yrrolizidinealkaloids,* which are said to be hepatocarcinogens; a*ristolochic acid I*, said to bemutagenic and carcinogenic; *phorbol esters,* which are tumor promoters andvesicant to the skin; *carboxyactractyloside*, a deadly toxic compound; a*mygdalin*, 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 bacterialand 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). (Skibola and Smith, 2000).

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 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. *(Johanson, J.F. and Sonnenberg, A., 1990).*

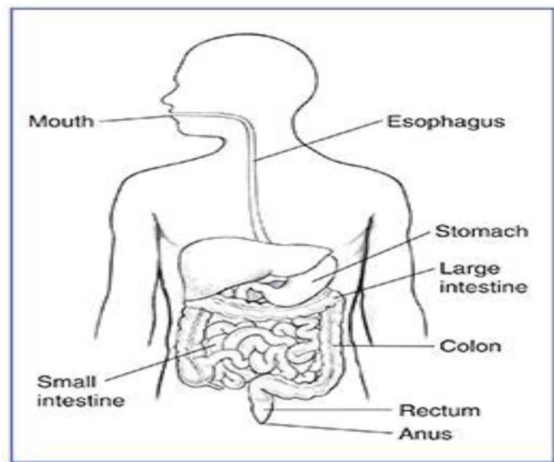

Figure 1.1: Human Gastrointestinal Tract *(Johanson, J.F. and Sonnenberg, A., 1990).*

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

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.

- 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. *(Johanson, J.F. and Sonnenberg, A., 1990).*

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. *(Blenkinsopp, A., Paxton, P. and Blenkinsopp, J., 2005).*
- 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).*

1.2.6. Functional GI Disorders

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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. *(Blenkinsopp, A., Paxton, P. and Blenkinsopp, J., 2005).*

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.

1.2.10. 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).

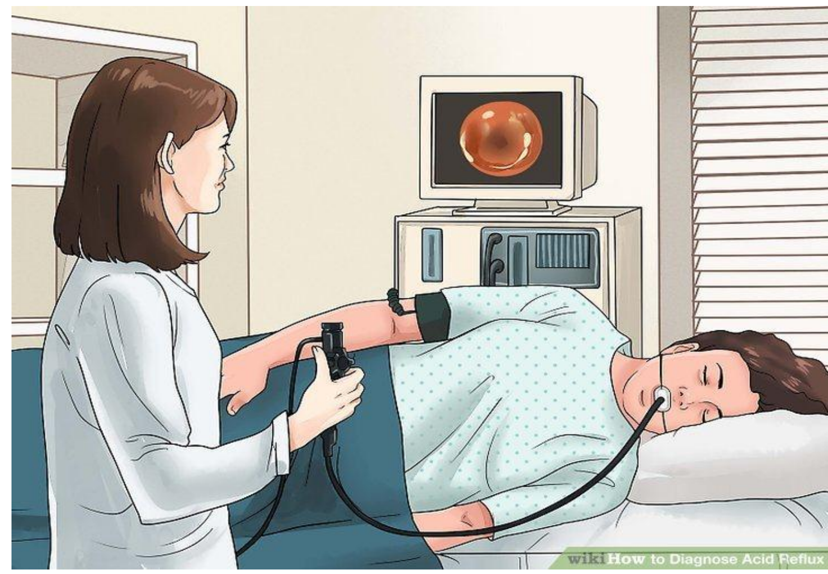

Figure 1.2: Flexible sigmoidoscopy (*Cash, B.D., Schoenfeld, P. and Chey, W.D., 2002)*

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 potassiumtartarate, 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
$C_{22}H_{19}NO_4$

Fig 1.2.13:Bisacodyl *(Adams, W.J., Meagher, A.P., Lubowski, D.Z. and King, D.W., 1994)*

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.

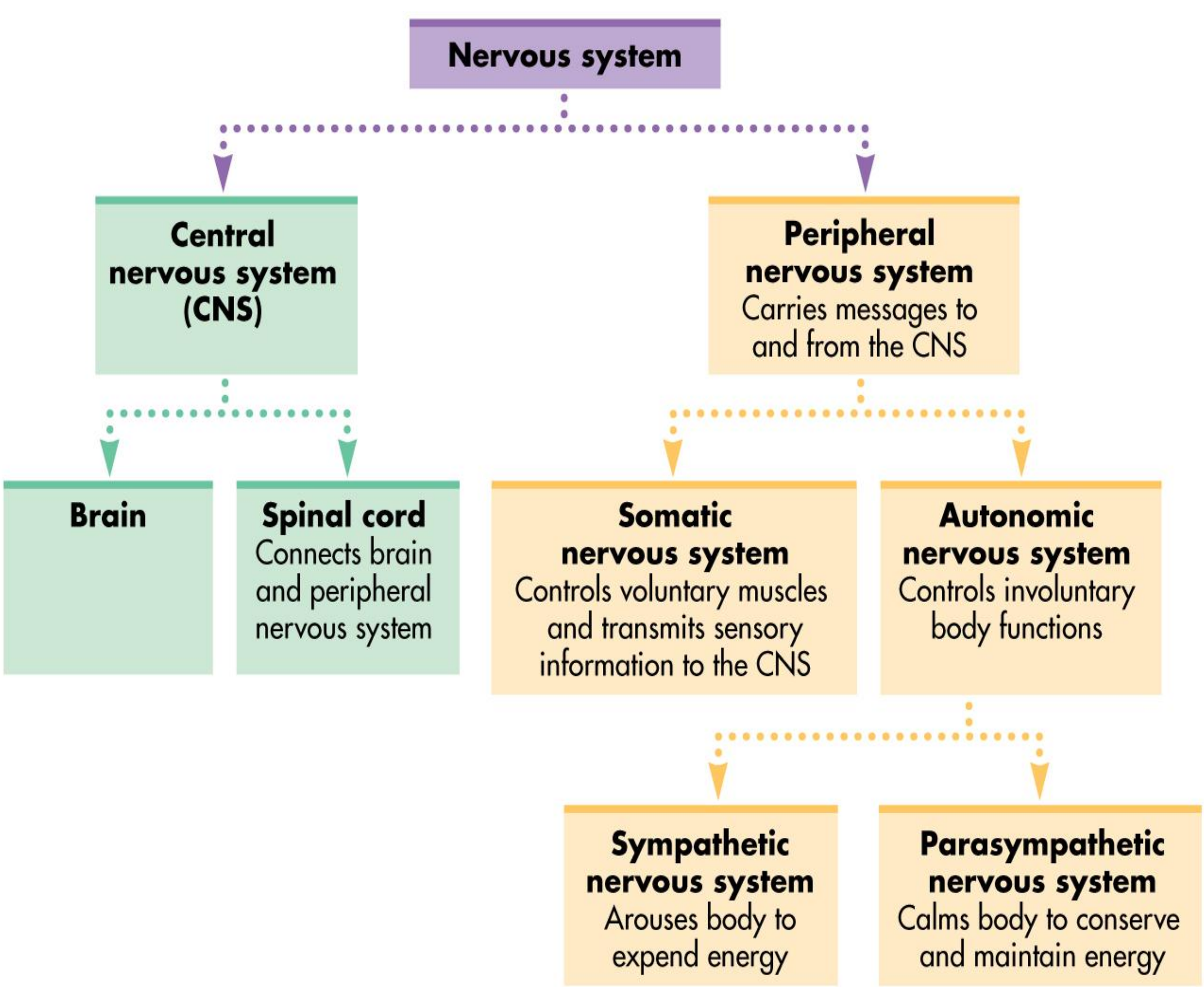

Figure1.3: Organization of the Human Nervous System. (*Reynolds, B.A. and Weiss, S., 1992).*

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)*

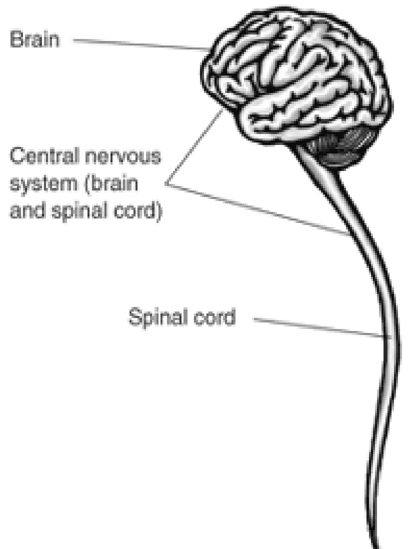

Figure1.4: Central Nervous System (*Reynolds, B.A. and Weiss, S., 1992*).

1.3.1.1. Parts of Central Nervous System

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

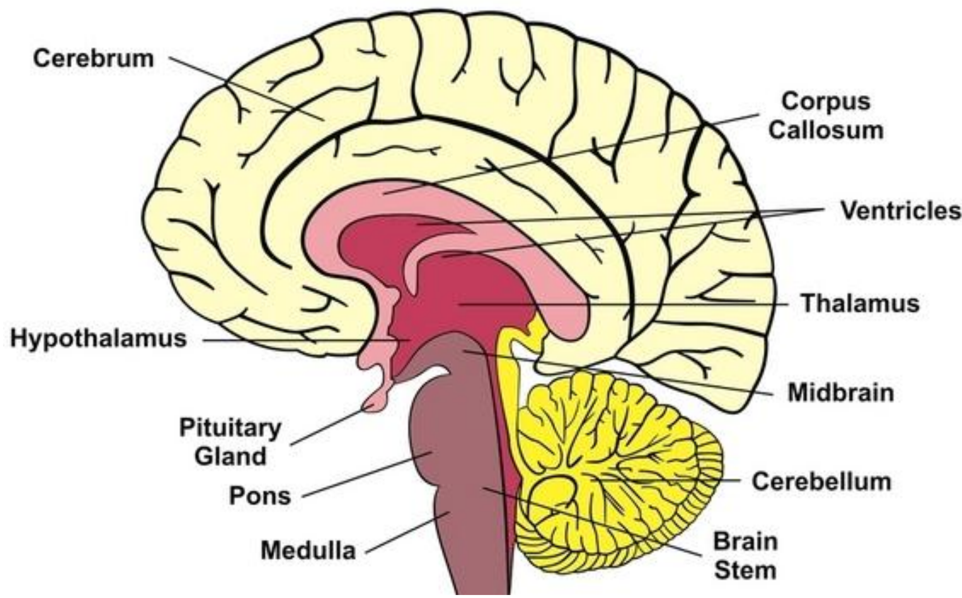

Figure 1.3.1.1: Human Brain (*Sunaert, S., Van Hecke, P., Marchal, G. and Orban, G.A., 1999*)

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.

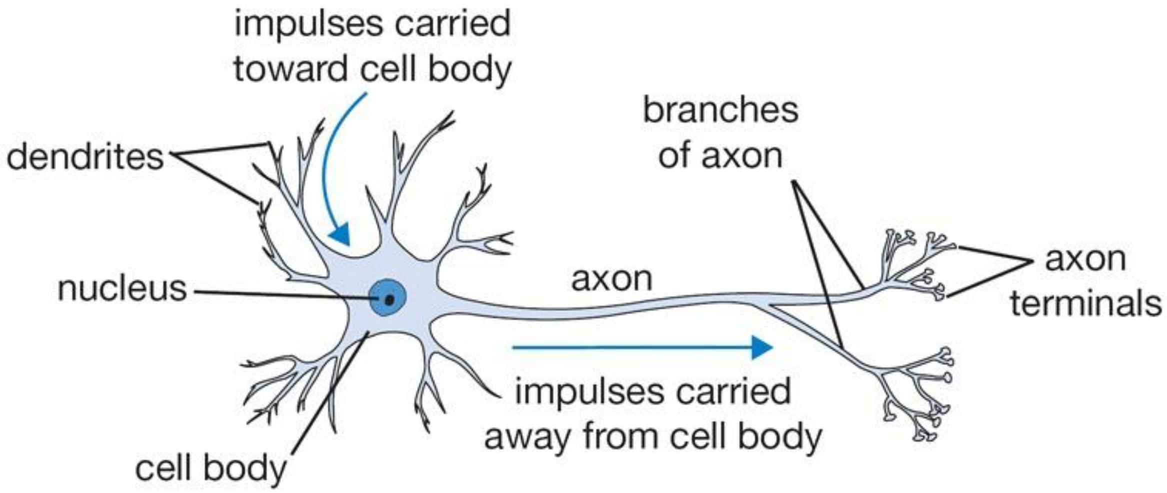

Figure 1.3.3: Neuron (*Sunaert, S., Van Hecke, P., Marchal, G. and Orban, G.A., 1999*)

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. (*Deckwerth, T.L. and Johnson, E.M., 1994*).

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. (*Deckwerth, T.L. and Johnson, E.M., 1994*).

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).*

1.4.3.1. Routes of administration

This term refers to the way in which drugs or compounds are introduced to animal’s orhumans. 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 15º 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. *(Howland and Mycek., 2006).*

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 thee 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. *(*Howland and Mycek, 2006).

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 bloods 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.

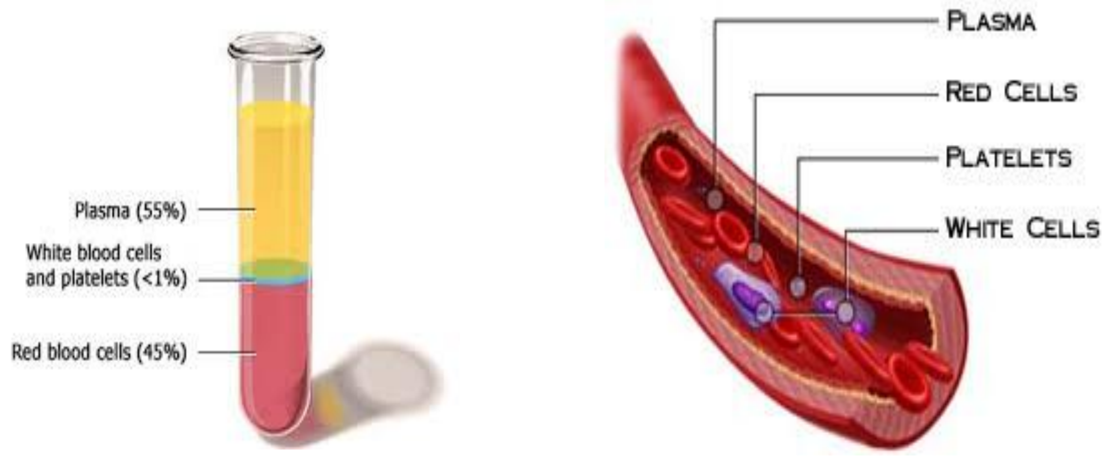

Figure 1.4.5.1.: Plasma of the Blood *(E Jonge, E. and Levi, M., 2001)*

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.

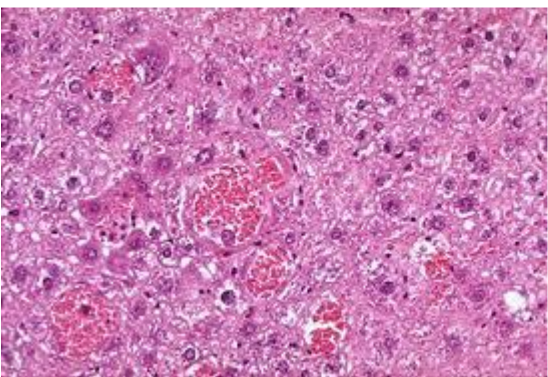

Figure 1.4.6*:* Red Blood cell *(E Jonge, E. and Levi, M., 2001)*

- **Normal range of RBC 8-16×10^6mm^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
- 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. *(Van Beaumont, W., 1972).*

Lower than Normal Hematocrit

- Infants without adequate iron intake
- 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. (Tonnesen, 1986).

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.

MCHC = $H_b / H_{ct} \times 100$

Normal range: 32-36 g/dL

When the MCHC is abnormally low they are called hypochromic, and when the MCHC is abnormally high, hyperchromic. *(Erslev, A.J. and Atwater, J., 1963)*

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. *(Erslev, A.J. and Atwater, J., 1963)*

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

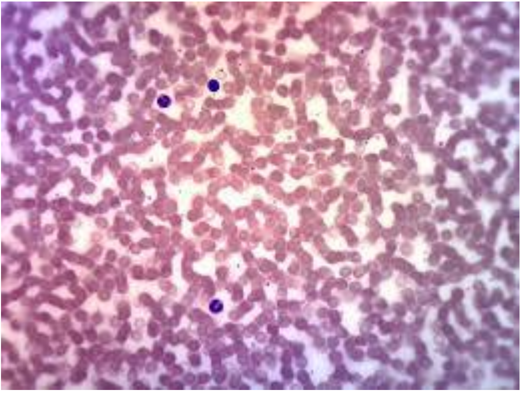

Figure 1.4.7: White Blood Cells *(Erslev, A.J. and Atwater, J., 1963)*

Normal range of WBC: 3-7×10^{3}mm^3

Different count of WBC

Neutrophils: Approx 70%, it s 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 helps 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 narrow. 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: 1000-1600×103mm3 (*Ganong, 2003*)**.**Normal range of platelet: 1000-1600×10^{3}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.

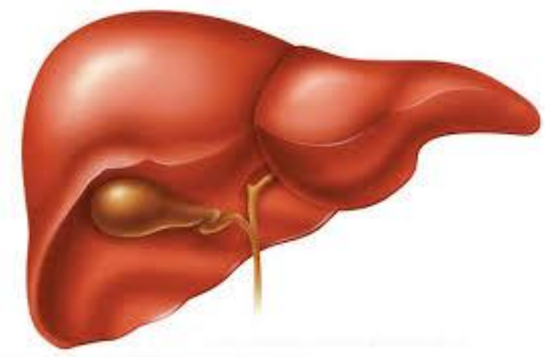

Figure 1.4.10: Liver *(Bogliolo, L., 1957.)*

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.

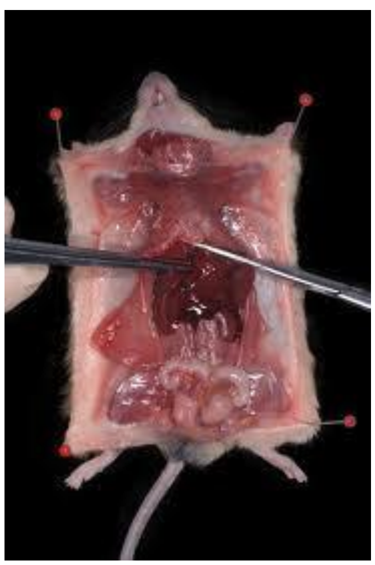

Figure 4.1.10: Anatomy of mice (*Bogliolo, L., 1957.)*

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 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 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. *(Green, D.E., Leloir, L.F. and Nocito, V., 1945)*

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 *(Green, D.E., Leloir, L.F. and Nocito, V., 1945)*

1.5. Thrombolysis

Thrombolytic Activity

Medicinal plants contain different therapeutic agents which may have thrombolytic activity. Atherothrombotic diseases such as myocardial or cerebral infarction occur as serious impacts of the thrombus formed in blood vessels. Acute coronary syndrome (ACS) patients are at increased risk of cardiovascular events, despite optimal antiplatelet medication. Thrombotic events depend on the propensity for thrombus formation and the efficacy of endogenous thrombolytic activity in preventing lasting arterial occlusion. *(Saraf et al., 2009).*

Various thrombolytic agents are used to dissolve the clots that have already formed in the blood vessels. One of the major causes of blood circulation problem is the formation of blood clots. Thrombi or emboli can lodge in a blood vessel and block the flow of blood in that location depriving tissues of normal blood flow and oxygen. This can result in damage, destruction or

even death of the tissues (necrosis) in that area. Thrombolytic therapy reduces mortality. A blood clot (thrombus) is developed in the circulatory system due to failure of hemostasis causes vascular blockage which formed from fibrinogen by thrombin and is lysed by plasmin, which is activated from plasminogen by tissue plasminogen activator (tPA). Fribrinolytic drugs has been used to dissolve thrombi in acutely occluded coronary arteries there by to restore blood supply to ischaemic myocardium, to limit necrosis and to improve prognosis (*Laurence & Bennett, 1992).*

Commonly used thrombolytic agents are alteplase, anistreplase, streptokinase, urokinase and tissue plasminogen activator (tPA) to dissolve clots (*Anwar et al., 2011*).

Streptokinase is an antigenic thrombolytic agent used for the treatment of acute myocardial infarction.It reduces mortality as effectively as the nonantigenic altreplase in most infarct patients while having the advantages of being much less expensive.Tissue-type Plasminogen activator (tPA) is generally preferred as being effective and safer than either urokinase or streptokinase type activators *(Khanet al., 2011).*

Streptokinase forms a complex with plasminogen (Figure 14) which then converts plasminogen to plasmin. Plasmin breaks down clots as well as fibrinogen and other plasma proteins (*Banerjee et al., 2004*).

All available thrombolytic agents still have certain significant shortcomings, including the need for large doses to be maximally effective, limited fibrin specificity and a significant associated bleeding tendency which cause serious and sometimes fatal consequences. All thrombolytic agents work by activating the enzyme plasminogen that clears the cross-linked fibrin mesh (*Khan et al., 2011*).

Thrombolytic drugs are widely used for the management of cerebral venous sinus thrombosis patients.During the last three decades of the 20th century, research activity in antithrombotic field was devoted to compounds showing anti-aggregator potency. Several drugs were explored,but except aspirin, among the huge number of synthetic molecules tested, only very few of them found a clinical use (*Dupin et al., 2002*).

1.6.Antioxidant

An antioxidant is a molecule stable enough to donate an electron to a rampaging free radical and neutralize it, thus reducing its capacity to damage. These antioxidants delay or inhibit cellular damage mainly through their free radical scavenging property (*Halliwell B., 1995).*

These low-molecular-weight antioxidants can safely interact with free radicals and terminate the chain reaction before vital molecules are damaged. Some of such antioxidants, including glutathione, ubiquinol and uric acid, are produced during normal metabolism in the body (Shi HL., 1999). Other lighter antioxidants are found in the diet. Although there is several enzymes system within the body that scavenges free radicals, the principle micronutrient (vitamins) antioxidants are vitamin E (α-tocopherol), vitamin C (ascorbic acid) and B-carotene (*Levine M. et al., 1991*). The body cannot manufacture these micronutrients, so they must be supplied in the diet.

1.6.1 Antioxidant Defense System

Antioxidants act as radical scavenger, hydrogen donor, electron donor, peroxide decomposer, singlet oxygen quencher, enzyme inhibitor, synergist and metal-chelating agents. Both enzymatic and nonenzymatic antioxidants exist in the intracellular and extracellular environment to detoxify ROS (*Frie B. et al., 1988*).

1.6.2. Mechanism of Action of Antioxidants

Two principle mechanisms of action have been proposed for antioxidants (Rice-Evans CA, Diplock AT., 1993). The first is a chain- breaking mechanism by which the primary antioxidant donates an electron to the free radical present in the systems. The second mechanism involves removal of ROS/reactive nitrogen species initiators (secondary antioxidants) by quenching chain-initiating catalyst. Antioxidants may exert their effect on biological systems by different mechanisms including electron donation, metal ion chelation, co-antioxidants, or by gene expression regulation (*Krinsky NI., 1992*).

1.6.3. Types of Antioxidants

Enzymatic:

Cells are protected against oxidative stress by an interacting network of antioxidant enzymes. These are: Superoxide dismutase, Catalase, Glutathione systems.

Non-Enzymatic:

Although there are several enzyme systems within the body that scavenge free radicals, the principle micronutrient (vitamin) antioxidants are: Ascorbic acid, Glutathione, Melatonin, Tocopherols and tocotrienols (Vitamin E), Uric acid

1.6.4 Plants as a Source of Antioxidants

Synthetic and natural food antioxidants are used routinely in foods and medicine especially those containing oils and fats to protect the food against oxidation. There are a number of synthetic phenolic antioxidants, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) being prominent examples. These compounds have been widely uses as antioxidants in food industry, cosmetics and therapeutic industry. However, some physical properties of BHT and BHA such as their high volatility and instability at elevated temperature, strict legislation on the use of synthetic food additives, carcinogenic nature of some synthetic antioxidants, and consumer preferences have shifted the attention of manufacturers from synthetic to natural antioxidants (Papas AM, 1993). In view of increasing risk factors of human to various deadly diseases, there has been a global trend toward the use of natural substance present in medicinal plants and dietary plats as therapeutic antioxidants. It has been reported that there is an inverse relationship between the dietary intake of antioxidant-rich food and medicinal plants and incidence of human diseases. The use of natural antioxidants in food, cosmetic and therapeutic industry would be promising alternative for synthetic antioxidants in respect of low cost, highly compatible with dietary intake and no harmful effects inside the human body. Many antioxidant compounds, naturally occurring in plant sources have been identified as free radical or active oxygen scavengers (Brown JE,1998).

Attempts have been made to study the antioxidant potential of a wide variety of vegetables like potato, spinach, tomatoes, and legumes (Furuta S, 1997). There are several reports showing antioxidant potential of fruits (Wang H, 1996).

Strong antioxidants activities have been found in berries, cherries, citrus, prunes, and olives. Green and black teas have been extensively studied in the recent past for antioxidant properties since they contain up to 30% of the dry weight as phenolic compounds (*Lin JK. et al, 1998*).

Chapter 02

Introduction to the plant

2. Introduction to the plant

2.1. Vernacular names:

Table 1: Showing the vernacular names of *Aglaonema hookerianum* Schott in different regions (*Islam et al., 2012*)

Region/Tribal name	*Vernacular names*
Bangla	Habinishak, Patabahar
Chakma	Gach Petic, Shakkosala, Sikkachala
Tanchangya	Shackkatola
Marma	Chekhow, Khaichcha Parabol, Meggey
Tripura	Hatharikhiethok
Khumi	Lykho

2.1.2 Taxonomy

The scientific classification of *Aglaonema hookerianum* Schott is

Kingdom: Plantae

Phylum: Tracheophyta

Class: Liliopsida

Order: Alismatales

Family: Araceae

Genus: Aglaonema

Species: Aglaonema hookerianum

2.1.3. Botanical name/Synonym: *Aglaonema clarkei* Hook.f.

2.1.4. Scientific name: *Aglaonema hookerianum* Schott

2.1.5. Group: Monocot (Malek, I., Mia, N., Mustary, E.,2014).

2.1.6. Plant Family: Araceae

2.2. The family of Aroids or Araceae

The Araceae, or aroids, is a family of herbaceous monocotyledons with 125 genera and about 3750 species Araceae is a family of monocotyledonous flowering plants in which flowers are borne on a type of inflorescence called a spadix. The spadix is usually accompanied by, and sometimes partially enclosed in, a spathe or leaf-like bract. Also known as the arum family, members are often colloquially known as aroids. The family is predominantly tropical in distribution, with 90% of genera and c. 95% of species restricted to the tropics. Although the greatest number of species originate in South America (including the two largest genera, Anthurium and Philodendron with over 1500 species between them, the tropics of South East Asia are also very rich, with the large and horticulturally important genera Alocasia and Amorphophallus (*Nicolson, 1969*).

The Araceae contains several well-known cultivated foliage and flowering plants. Members of the family are highly diverse in life forms, leaf morphology, and inflorescence characteristics. Life forms range from submerged or free-floating aquatics to terrestrial (sometimes tuberous), and to epiphytic or hemiepiphytic plants or climbers. Leaves range from simple and entire to compound and highly divided, and may be basal or produced from an aerial stem. The family

Araceae is defined by bearing small flowers on a fleshy axis (spadix) subtended by a modified leaf (spathe) (Nicolson, 1969).

In some genera the spathe is very conspicuous and brilliantly coloured (e.g., many Anthurium species) while in others the spathe is small and leaf-like (e.g., many Pothosspecies). In the North American genus Orontium the spathe is so reduced that it appears to be absent altogether and in Gymnostachys, a peculiar genus restricted to eastern Australia, debate continues as to whether a spathe is in fact present or, indeed, if Gymnostachys might be better removed altogether from the aroids. The behaviour of the spathe varies from genus to genus. In some (e.g., Cryptocoryne) the spathe completely encloses the spadix, while in others the spathe reflexes to leave the spathe clearly visible(e.g., most Anthurium, Spathiphyllum). In some genera the spathe is shed as soon as the inflorescence reaches anthesis, either falling completely (e.g., Rhaphidophora) or partially (e.g., Schismatoglottis). The spathe ranges in size and shape from 5mm long and simple in Homalomena humilis to the fluted and pleated vase 1m wide and 1.5m tall found in Amorphophallus titanium (Nicolson, 1969).

The sex of the individual flowers and their arrangement on the spadix are among the characters used to define taxonomic groups. Depending on the genus the spadix may bear either unisexual or bisexual flowers. If bearing bisexual flowers there are uniformly arranged over the spadix. Almost without exception bisexual flowers are subtended by reduced tepals termed a perigon. If unisexual, the flowers are usually arranged with the females at the base of the spadix occasionally terminated by a sterile appendix. In the genus Arisaema individual inflorescences are usually either male or female. The sex of the inflorescence in Arisaema is governed by the age of the plant, its health, and the type of conditions in which it is growing (Kumar, 2015).

Young plants or mature plants in poor condition or growing in a less than ideal habitat will produce male inflorescences. The ability to alter the sex of the inflorescence in this way is termed paradioecy. Unisexual flowers are almost without exception naked. i.e.,lacking a perigon (Nicolson, 1969).

The most recent technical account for the genera is The Genera of Araceae (*Mayo,Bogner & Boyce, 1997*) while a species checklist for the family, World checklist and bibliography of Araceae (and Acoraceae) by Govaerts, Frodin etal., appeared in 2002.Aside from floristic accounts and taxonomic treatments the best non-technical account is that of Bown (2000). The classic work on the genus Arum, Lords and Ladies (Prime,1960), is essential reading for anyone wanting to understand the pollination strategy employed by many monoecious aroid taxa (Nicolson, 1969).

All Araceae studied to date display insect pollination. Many,notably *Amorphophallus* have evolved to be pollinated by insects attracted to dung or carrion (saproentomophily). Many tropical species have inflorescences where pollination has evolved in conjunction with bees, wasps and beetles. In species of *Philodendron* investigated to date large dynastid scarab beetles are attracted to the inflorescences and appear to be the main pollinators (Gottsberger & Amaral, 1984).Many aroids attract pollinators by odor. Inflorescence odors include dung, carrion, rotting fruit, old socks, semen, bad breath, beer, spearmint, cheap sweets and cinnamon (*Nicolson, 1969*).

Several general have inflorescences that heat up considerably during anthesis, often by as much 20°C above the ambient temperature and often producing at the same time a strong, foul odor. Some genera also offer potential pollinators food in the form of fat bodies (*Dieffenbachia*), sugar solutions (many *Arum* species) or oil droplets (*Amorphophallus*).(*Nicolson, 1969*).Within the Aracae, genera such as *Alocasia*, *Arisaema*, *Caladium*, *Colocasia*, *Dieffenbachia*, and *Philodendron* contain calcium oxalate crystals in the form of raphides. When consumed, these may cause edema, vesicle formation and dysphagia accompanied by painful stinging and burning to the mouth and throat, with the symptoms occurring for up to two weeks (Nicolson, 1969).

2.3. Macroscopic/Morphological characteristics

It is an annual herb. Stem is erect, 40-50 cm or more, 1.5-2.0 cm thick. Internodes are 1.5-3.0 cm long. Petioles are 14-24 cm long, 0.7-0.9 (1.2) times as long as the leaf-blade. Sheaths

membranous, (4) 8-15 cm long, (0.2) 0.5-0.6 (0.8) times as long as the petiole. Leaf-blade ovate to elliptic or lanceolate to narrowly elliptic, 20-27 cm long (5.3) 7-12cm wide, length/width ratio 1 : 2.0-2.9 (3.8) ; base often unequal, rounded, obtuse or broadly acute, rarely acute; apex often apiculate, acuminate to gradually or suddenly long acuminate (acumen to 2.1 cm long from point of 1 cm blade width to apex) ; variegationnone; venation weakly to strongly differentiated into 7-13 primary lateral veins which diverge from the midrib at (30') 40‖-50‖. Peduncles 1-3 together, 10-21 cm long. Spathe 3.7-6.0 cm long, decurrent for (0.6) 1-1.5 (2) cm. Stipe none. Spadix thin-cylindric, 2.5-4.0 cm long, equaling to 0.8 cm short of spathe apex; pistillate portion 0.3-0.6 cm long, attached to spathe, pistils ca. 10-15; staminate portion 2.0-3.7 cm long, 0.3-0.6 cm thick. Fruits red, large when ripe, (1.7) 2-3 cm long, (0.7) 0.9-1.4 cm thick (*Nicolson, 1969*).

2.4. Distribution

Darjiling, Assam, Bhutan, and Myanmar. In Bangladesh, it is found in the forests of Sylhet, Chittagong and Chittagong Hill Tracts. (Motaleb, 2013*)*

2.5.Distinguishing Features

The distinctive characteristics of *Agalonema hookerianum* are (Nicolson, 1969)-

- sessile spadix,
- spadix equaling the spathe
- large fruits,
- a long peduncle (compared with *A. ovatum* and *A. modestum)*,
- restriction to the above mentioned location or distribution

2.6. Habitat

Below 1000 m, in deep shade of forest receiving more than 80 inches of annual rainfall (*Nicolson, 1969).*

2.7 Flowering & Fruiting time

June-July, probably influenced by the onset of the summer monsoon. Fruit berry, bright red (*Nicolson, 1969*).

2.8 Propagation: Propagated by rhizomes (Nicolson, 1969).

2.9 Chemical constituents: Not known (Nicolson, 1969).

2.10. Local Uses

Different parts of this plant are applicable for various treatments-

Table 2: Local uses of *Aglaonema hookerianum* Schott in different regions of Bangladesh *(Biozid et al., 2015; Rahman et. al., 2007; Motaleb, 2013)*

Plant part	Tribe	Local use
root	Chakma	The sap of the root is taken orally for the treatment of conjunctivitis and constipation
leaf	Tanchangya A	A leaf extract is taken and a paste of the leaves is applied to the whole body for the treatment of hysteria people in Bangladesh
Spathe	Khumi, Marma and Tripura	Extracted spathe juice (two table spoon) taken orally

Fig **2.2**:leaves (*Uddin and Rahman, 2006*).

2.11 Medicinal uses

The species is used in the treatment of cirrhosis, flatulence, hyper acidity (gastritis) and tetanus (*Uddin and Rahman, 2006*).

2.12 Other uses

It is also used as vegetable. Sometimes it has been used as ornamental plants (*Motaleb,2013).*

2.13.Toxicology

- **Oxalates:** The juice or sap of the plant contains oxalate crystals. These needleshaped crystals can irritate the mucous membranes in skin, mouth, tongue, and throat, resulting in throat swelling, breathing difficulties, burning pain, and stomach upset (*Islam et al., 2012).*

- **Dermatitis:** The juice of the plant may cause a skin rash or irritation (*Islam et al.,2012*).

Table 3: Other species under Aglaonema

Species	
Aglaonema brevispathum	***Aglaonema ovatum***
Aglaonema chermsiriwattanae	***Aglaonema philippinense***
Aglaonema commutatum	***Aglaonema pictum***
Aglaonema cochinchense	***Aglaonema pumilum***
Aglaonema cochinchinense	***Aglaonema rotundum***
Aglaonema cordifolium	***Aglaonema tenuipes***

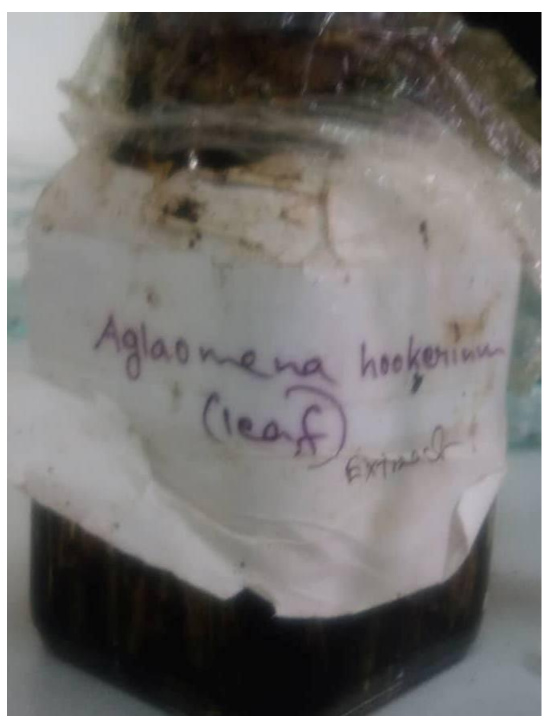

Fig**2.3**: *Aglaonema hookerianum (Extract)*

Fig**2.4**: *Aglaonema hookerianum (Leaf)*

Chapter 03

Literature Review

3. Review of literature

3.1 Polyhydroxyalkaloids in the Aroid Tribes Nephthytideae and Aglaonemateae: Phytochemical Support for an Intertribal relationship

In another survey of polyhydroxyalkaloids, living and herbarium material was used in species of 52 genera of Araceae which revealed the existence 2,5-dihydroxymethyl-3,4 dihydroxypyrrolidine (DMDP) and α-homonojirimycin (HNJ) in leaves of Aglaonema Schott. Levels were high in living plants, ranging from 0.1 to 1% dry weight DMDP and 0.04 to 0.6% HNJ. α-3,4-di-epihomonojirimycin, an isomer of HNJ, were also present *(Kite et al., 1997).*

3.2 Homonojirimycin Isomers and Glycosides from *Aglaonema treubii*

From a study performed on a 50% aqueous ethanol extract of *Aglaonema treubii* found to be potent in inhibiting α-glucosidase, several compounds were revealed when treated to various ion-exchange column chromatographic steps. The compounds were 2(*R*),5(*R*)-bis(hydroxymethyl)-3(*R*),4(*R*)-dihydroxypyrrolidine (1), α-homonojirimycin (2), β-homonojirimycin (3), α-homomannojirimycin (4), β-homomannojirimycin (5), α-3,4-di-*epi*-homonojirimycin (6), 7-*O*-β-d-glucopyranosyl-α-homonojirimycin (7), and 5-*O*-α-d-galactopyranosyl-α-homonojirimycin (8). Compounds 1 and 2 are known inhibitors of various α-glucosidases. Compounds 6 and 8 are new natural products.Compounds 3−5 and 7 have been chemically synthesized previously, but this report is known to be the first incidence of their natural occurrence. (*Asano et al., 1997*).

3.3 Antihyperglycemic Effects of N-Containing Sugars from *Xanthocercis zambesiaca*, *Morus bombycis*, *Aglaonema treubii*, and *Castanospermum australe* in Streptozotocin-Diabetic Mice

This study evaluated eight structurally related nitrogen-containing sugars, fagomine (1), 4-*O*-β-d-glucopyranosylfagomine (2), 3-*O*-β-d-glucopyranosylfagomine (3), 3-epifagomine (4), 2,5-dideoxy-2,5-imino-d-mannitol (5), castanospermine (6), α-homonojirimycin (7), and 1-deoxynojirimycin (8) present in *Morus bombycis*, *Aglaonema treubii*, and *Castanospermum australe* for antihyperglycemic

effects in streptozotocin (STZ)-diabetic mice. Compounds 1, 2, 5, and 6 reduced the blood glucose level after intraperitoneal injection of 150 μmol/kg. Due to compound 1 there was increased plasma insulin level in STZ-diabetic mice and potentiated the 8.3-mM glucose-induced insulin release from the rat isolated-perfused pancreas. Antihyperglycemic action may be partly contributed to the fagomine-induced stimulation of insulin release (*Nojima et al., 1998*).

3.4 Phytochemical screening, cytotoxicity and antibacterial activities of two Bangladeshi medicinal plants

The ethanolic extracts of leaves of *Aglaonema hookerianum* Schott (Family: Araceae) were investigated for the phytochemical screening and assaying cytotoxicity and antibacterial activities. The brine shrimp lethality bioassay of ethanolic extracts of *Aglaonema hookerianum* revealed cytotoxic activities with LC_{50} 5.25 (microg mL^{-1}) and LC_{90} 9.55 (microg mL^{-1}). Antibacterial activities of the extract were examined against some gram positive bacteria such as Bacillus subtilis, Bacillus megaterium and Staphylococcus aureus, also gram negative strains of Pseudomonas aeruginosa, Escherichia coli, Shigella dysenteriae, Salmonella typhi, Salmonella paratyphi and Vibrio cholerae. Using Agar disc diffusion method, antibacterial efficacy of the extract (500 microg $disc^{-1}$) was observed and compared with the zones of inhibition of Amoxicillin at concentration of 10 microg $disc^{-1}$. The extract produced significant antibacterial activity which indicates a useful source for the development of new potent antibacterial agents (*Roy et al., 2011).*

3.5 *In-vitro* anti-atherothrombosis activity of four Bangladeshi plants

Another study was conducted to investigate the thrombolytic activity of ethanol extracts of *Aglaonema hookerianum*. The study was carried out in-vitro with streptokinase being the reference standard and ethanol as negative control. The extract produced 11.18% of clot lysis with reference to streptokinase (81.08%) (*Islam et al., 2012).*

3.6 Shuktani – a new ethno-medico recipe among the Sylheti Bengali Community of Barak valley, Southern Assam, India

A survey was performed on the ethnobotanical study of Barak valley of Southern Assam for 10 years during which many new traditional prescriptions and recipes were known. Among such recipes Shuktani is one of the traditional and common recipes that is still in regular practice amongst the community of Barak valley of Sothern Assam. The preparation is being used for various diseases including stomach disorders and like diarrhoea, dysentery, indigestions, etc. and as a recipe for women as post parturition treatment, weakness and lactation. The recipe of Shuktani is prepared with the uses of 35 species of Angiosperms consisting of the leaves of 23, vegetative buds, fruits and seeds of 4 each, stems and flowers of 2 each of the plants in either liquid form or in powdered form which comprises *Aglaonema hookerianum* as one of the marked component (*Nath and G Maiti, 2012*).

3.7 Interior plants for sustainable facility ecology and workplace productivity

In this work, Burchett et al. examined the capacity of three plants including Aglaonema modestum for cleaning the environment from benzene, toluene, xylene and n-hexane, which are used as industrial solvents for furnishings. They showed that the contaminants concentrations decreased gradually below the detection limits of the gas chromatograph (*Mosaddegh et al., 2014).*

3.8 A comparative study of thrombolytic effects of methanolic extract of Bridelia stipularis and Aglaonema hookerianum leaf

Crude methanol extract of B. stipularis and A. hookerianum leaf was evaluated to compare the thrombolytic activities among them using the in vitro clot lysis model. Venous blood taken from five healthy workers was allowed to form clots. Then the clot was weighed and treated with the test samples from the plant extracts to disrupt the clots. The percentage of clot lysis determined from the weight of clot before and after treatment was compared with the streptokinase as the positive control and water as the negative control. Form the study, it was observed that B. stipularis and A. hookerianum showed 33.42 ± 3.37% and 24.72 ± 2.75% of clot lysis respectively. As the result was compared with the reference drug streptokinase

(63.54 ± 2.61%), it was found that A. hookerianum showed significant ($p < 0.001$) percentage of clot lysis but comparably less significant than the two plants (*Biozid et. al., 2015).*

3.9 Photocytotoxic pheophorbide-related compounds from *Aglaonema simplex*

In a screening program evaluated on the leaves and stems of Aglaonema simplex for new photosensitizers, five pheophorbide-related compounds were found. Compounds 1-3 and 5 are pheophorbide and hydroxy pheophorbide derivatives of chlorophyll a and b as shown by the detailed spectroscopic analyses. Compound 4 was isolated for the first time from the Araceae family which was identified as 15(1)-hydroxypurpurin-7-lactone ethyl methyl diester. An MTT-based short-term survival assay was applied on all five compounds which showed that they exhibited moderate-to-strong photocytotoxic activities towards human leukemia (HL60) and two oral squamous carcinoma cell lines.

Chapter-04

Materials and Methods

4.1. Plant Materials

4.1.1 Collection of plant

The plant was collected from Sylhet district of Bangladesh. A voucher specimen (Accession number: 39643) had been deposited at the Bangladesh National Herbarium. The proper time of harvesting or collecting is particularly important because the nature and the quantity of constituents very gently in some species according to the season.

4.1.2. Preparation of plant extraction

The leaf 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 *Aglaonema hookerianum* 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 4.1.: Rotary evaporator & crude extract in a bottle

4.2. Experimental Animals

Swiss albino 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±2.0°C and 12 h light: dark cycle). The animals were fed with standard diet and water ad libitum.

Figure 4.2.*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 treated per oral (p.o.) with increasing doses of 200mg/kg, 400 mg/kg & 800mg/kg of the plant *Aglaonema hookerianum* 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 intraperitonial (i.p.) injection of atropine (10 mg/kg) 15 min prior the administration of the extract.

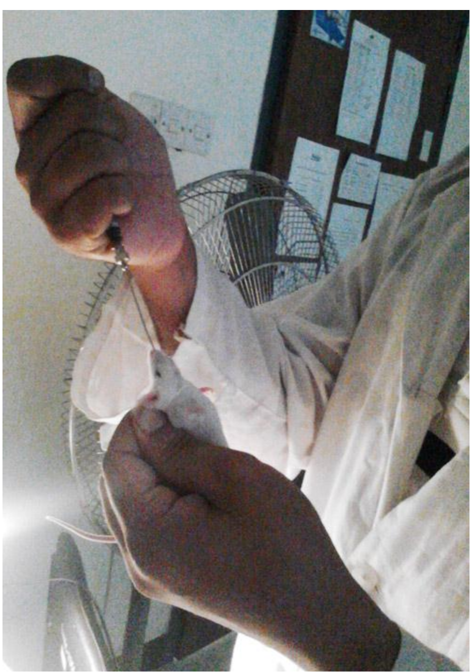

Figure 4.5. : Oral administration into mouse.

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 non-disposable
- 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 *Aglaonema hookerianum* 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.
- 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 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 of *Aglaonema hookerianum* the doses of 200,400 & 800mg/kg body weight orally. The floor of an open field of half square meter was divided in to 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 4.6.5.1.: Open Field 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
- Eppendroff 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 *Aglaonema hookerianum* 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 *Aglaonema hookerianum* a dose of 200, 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 2000 and 4000, 8000 mg/kg body weight, orally. The treated animals were kept under observation for 3 days, for mortality and general behavior. *(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 Eppendroff 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 (National Institute of Cancer Research & Hospital (NICRH), Dhaka, Bangladesh), 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(National Institute of Cancer Research & Hospital (NICRH), Dhaka, Bangladesh)

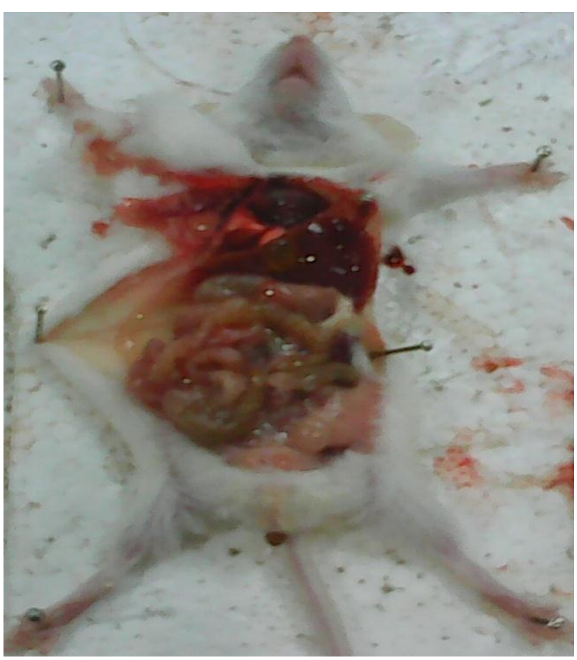

Figure 4.6. Mice Organ

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 5-7μ. Routine histopathology was performed (National Institute of Cancer Research & Hospital (NICRH), Dhaka, Bangladesh) by using the Haemotoxylin stain (*Paul, et.al., 2012).*

4.7.8. Statistical Analysis

Data obtained from pharmacological experiments are expressed as mean±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.

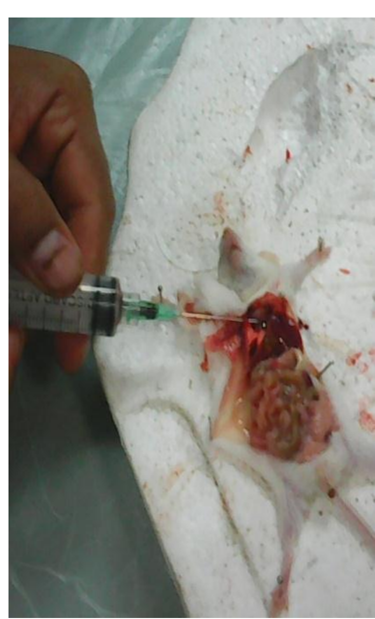

Figure 4.7. Collecting the blood and organ of mice

4.8. Antioxidant:

4.8.1 Total phenolic Content Assay

4.8.1.1. Introduction

The antioxidative effect is mainly due to phenolic components, such as flavonoids, phenolic acids, and phenolic diterpenes. The antioxidant activity of phenolic compounds is mainly due to their redox properties, it has been reported that there is an inverse relationship between the antioxidative status occurrences of human diseases.

In addition, antioxidant compounds which are responsible For Such antioxidants activity, could be isolated and then used as antioxidants for the prevention and treatment of free radical-related disorders.

4.8.1.2. Principle

In the alkaline condition phenols ionize completely. When Folin-Ciocalteu reagent is used in this ionized phenolic solution the reagent will readily oxidize the phenols. Usual color of Folin-Ciocalteu reagent is yellow and after the oxidation process the solution become blue. The intensity of the color change is measured in a spectrophotometer at 700nm. The absorbance value will reflect the total phenolic content of the compound.

4.8.1.3. Materials & Methods

Total phenolic content of crudeextracts was measured employing the method as described by Arpona Hira et al. (2013) involving Folin-Ciocalteu reagent as oxidizing agentand salicylic acid as standard.

Table 4.: Composition of Folin-Ciocalteu Reagent

Water	57.5ml
Lithium Sulfate	15.0mg
Sodium Tungstate Dihydrate	10.0mg
Hydrochloric Acid 25%	10.0mg
Phosphoric Acid 85 % solution in water	5.0mg
Molybdic Acid Sodium Dihydrate	2.5mg

4.8.1.4. Standard Curve Preparation

Salicylic acid was used here as standard. Different concentration of Salicylic acid solution were prepared having a concentration ranging from 10 mg/ml to 0.625mg/ml. 5.0 ml of Folin-Ciocalteu reagent (diluted 10 times with water) and 4.0 ml of Na_2CO_3 (7.5 % w/v) solution was added to 100µl of Salicylic acid solution. The mixture was incubated for 1 hour at room temperature. After 1 hour the absorbance was measured at 700 nm. After plotting the absorbance in ordinate against the concentration in abscissa a linear relationship was obtained which was used as a standard curve for the determination of the total phenolic content of the test samples.

4.8.1.5. Sample Preparation

10 mg of crude extract was taken and dissolved in 1ml of methanol (*Aglaonema hookerium)* and 1ml of ethanol (*Aglaonema hookerianum)* to get a sample concentration of 10mg/ml in every case.

4.8.1.6. Determination of Phenolic Content of Samples

100 µlsolution of crude extract mixed with 5.0 ml of Folin-Ciocalteureagent (diluted 10 times with water) and 4.0 ml of Na_2CO_3 (7.5 % w/v) solution. The mixture was incubated for 1 hour atroom temperature. After 1 hour the absorbance was measured at 765 nm. Using the absorbance of the sample, total phenolic content is measured by using following equation ---

$$T = \frac{C \times V}{M} \text{ mg}$$

Where,

T = Total phenolic content

C = x (Concentration from linear regresson equation)

V = Volume of sample

M = Mass of sample

4.8.2.. DPPH Radical-Scavenging Activities

4.8.2.1. Introduction

There is considerable recent evidence that free radical induce oxidative damage to biomolecules. This damage causes cancer, aging, neurodegenerative diseases, atherosclerosis, malaria and several other pathological events in living organisms. Antioxidants which scavenge free radicals are known to possess an important role in preventing these free radical induced-diseases. There is an increasing interest in the antioxidants effects of compounds derived from plants, which

could be relevant in relations to their nutritional incidence and their role in health and diseases. A number of reports on the isolation and testing of plant derived antioxidants have been described during the past decade. Natural antioxidants constitute a broad range of substances including phenolic or nitrogen containing compounds and carotenoids.

Lipid peroxidation is one of the main reasons for deterioration of food products during processing and storage. Synthetic antioxidant such as *tert*-butyl-1-hydroxytoluene (BHT), butylated hydroxyanisole (BHA), propyl gallate (PG) and tert-butylhydroquinone (TBHQ) are widely used as food additives to increase self life, especially lipid and lipid containing products by retarding the process of lipid peroxidation. However, BHT and BHA are known to have not only toxic and carcinogenic effects and humans, but abnormal effects on enzyme systems. Therefore, the interest in natural antioxidant, especially of plant origin, has greatly increased in recent years.

4.8.2.2. Principle

The free radical scavenging activity of plant extracts against stable DPPH (2,2-diphenyl-2-picrylhydrazyl) was determined spectrophotometrically. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it was reduced. The changes in color (from deep violet to light yellow) were measured at 517 nm on a UV-visible light spectrophotometer.

4.8.2.3. Material and Method

DPPH was used to evaluate the free radical scavenging activity of crude extracts was measured employing the slightly method described by Arpona Hira et al. (2013) involving DPPH as oxidizing agent and Ascorbic acid as standard.

4.8.2.4. Preparation of DPPH Solution

A dry 250ml conical flask was cleaned and covered with an aluminium foil protect its contents from light. Accurately weighted 2mg of DPPH placed in conical flask and 50ml methanol was added to prepare 0.1mmol/L or 40µg/ml DPPH solution.

4.8.2.5. Preparation of Sample Solution

Accurately weighted 20 mg of plant extract was taken into a vial and 2ml of methanol was added and the concentration of final solution is 10μg/μl ten test tube were taken and covered with an aluminium foil protect its contents from light and these test tubes were marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from 0.98 μg/ml to 500 μg/ml. The test was done three times.

4.8.2.6. Preparation of Standard Solution

Accurately weighted 20 mg of ascorbic acid as standard was taken into a vial and 2 ml of distilled was added and the concentration of final solution is 10μg/μl ten test tube were taken and covered with an aluminium foil protect its contents from light and these test tubes were marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from 0.98 μg/ml to 500 μg/ml. The test was done three times.

4.8.2.7. Measurement of DPPH Radical Scavenging Activity

2ml of a methanol solution of the extract at different concentration were mixed with 2ml of a DPPH methanol solution and this mixture was vigorously shacked and left at 25^0C for 60 minutes in the dark. After 60 minutes reaction period at room temperature in dark place the absorption was measured at 517nm of methanol as blank by UV spectrophotometer.

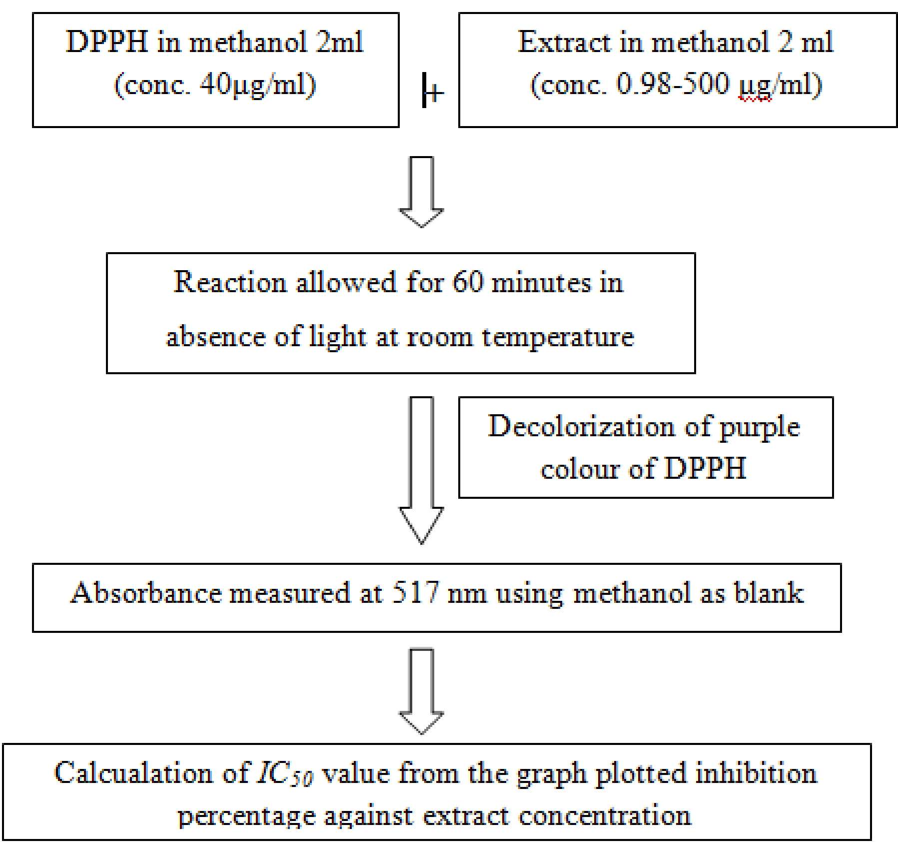

Figure 4.8.: Schematic representation of the method of assaying free radical scavenging activity.

4.8.3. Reducing Power Assay

Introduction

Free radicals are types of Reactive Oxygen Species (ROS), which include all highly reactive, oxygen-containing molecules. Types of ROS include the hydroxyl radical, the super oxi de anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, an d various lipid eroxides. These free radicals may either be produced by physiological or biochem ical processes or by pollution and other endogenous sources. All these free radicals are capable o f reacting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, resulting in cellular damage

Antioxidants prevent the human system by neutralizing the free radicals interactively and synergi stically. Plants arerich source of free radical scavenging molecules such as vitamins, terpenoids,

phenolic acids, lignins, stilbenes, tannins, flavanoids, quinones, coumarins, alkaloids, amines, bet alains and other metabolites which are rich in antioxidant activity.

Principle

Substances, which have reduction potential, react with potassium ferricyanide (Fe3+) to form potassium ferrocyanide (Fe2+), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm.

Material and Method

Reducing power assay of crude extracts was measured employing the method as described by Arpona Hira et al. (2013) involving Phosphate Buffer (2.5ml, 0.2M, pH 6.6), 1% Potassium Ferricyanide [$K_3Fe(CN)_6$], 10% Trichloroacetic acid 0.1% $FeCl_3$and Ascorbic acid as standard.

Preparation of Sample Solution

Accurately weighted 20 mg of plant extract was taken into a vial and 2ml of methanol was added and the concentration of final solution is 10µg/µl ten test tubes were taken and marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from0.98µg/ml to 500 µg/ml. The test was done triplicate.

Preparation of Standard Solution

Accurately weighted 20 mg of ascorbic acid as standard was taken into a vial and 2 ml of distilled was added and the concentration of final solution is 10µg/µl ten test tube were taken and marked for different concentration. A serial dilution is carried out to prepare 10 different concentration of each sample and the final concentration ranging from0.98 µg/ml to 500 µg/ml. The test was done triplicate.

Procedure

1ml of stock mixture (concentration 0.98 µg/ml to 500µg/ml) is mixed with 1ml of distilled water added with 2.5ml of Phosphate Buffer and 2.5ml of 1% Potassium Ferricyanide. The reaction mixture is incubated at 50°C for 20minute. After incubation 10% Trichloroacetic acid is added. The mixture is centrifuged for 10min at 3000rpm. After centrifugation Upper layer was taken (2.5ml) dissolved with 2.5ml distilled water and 0.5ml of $Fecl_{3.}$ Absorbance was measured at 700nm.

4.9. In Vitro Thrombolytic Activity Test

4.9.1 Materials

4.9.1.2 Chemicals Equipments Glass apparatus

Saline (0.95% NaCl) ,Streptokinase (stk , incepta pharmaceuticals ltd) Balance, Vortex mixture, Eppendorf tube, Incubator ,Test tubes, Beaker, Acetone Micropipette (Eppendorff, Germany)

4.9.1.3 Method (*Khan et al., 2011*)

4.9.1.3.1 Preparation of stock

5 ml saline was added to streptokinase vial (15, 00,000 I.U) and mixed properly.

4.9.1.3.2 Preparation of extracts solutions

0.1 gm of n-hexane, ethyl acetate and methanol extracts of *Aglaonema hookerianum* leaves were weighed and suspended in 10 ml of saline (Figure 32) and it was shaked vigorously on vortex mixture. It was kept for overnight. After that the solutions were filtered and the filtrate was collected.

4.9.1.3.3 Preparation of blood sample

- Venous blood was collected from healthy human volunteers (n = 10) without having of oral contraceptives or anticoagulant therapy where male = 5 and female = 5. 500 µl of blood was transferred to previously weighed in 50 Eppendorf tubes.

- The tubes then kept for incubation to the incubator for 2 h at 37℃. After clot formation , serum was removed completely without disturbing the clot .
- Again weight was taken of each tube with clot. Clot weight was determined for each tube separately.
- Clot weight = wt. of clot containing tube – wt. of tube alone

4.9.2 Procedure

- 100 µl of extract solution was added to each tube containing clot
- For positive control 100 µl of streptokinase and for negative control 100 µl of normal saline was added.
- Again all tubes were incubated for 90 min at 37℃ and it was observed for clot lysis.
- After incubation release fluid was removed carefully (Figure 36). Again each tubes weighed was taken to observe the difference in weight after clot disruption.
- Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis as shown below:

$$\%\text{ of clot lysis} = \frac{\text{clot wt.} - \text{wt. of released clot}}{\text{clot wt.}} \times 100$$

Statistical analysis: Statistical comparisons were performed with Student's 't' tests using Microsoft Excel 2007. A p value of 0.001 or less was considered to be significant. Mean values ± S.D. were calculated for the parameters where applicable.

Chapter 05

Result & Discussion

5. Result and Discussion

5.1. *Aglaonema hookerianum* (Leaf):

Crude extract of *Aglaonema hookerianum* (Leaf) 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 *Aglaonema hookerianum* (Leaf) Table-5.1 and figure-5.1 show the laxative effect by charcoal meal GI transit test of the crude extract of *Aglaonema hookerianum* 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 table-1. 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 *Aglaonema hookerianum* were used for the gastrointestinal transit test.

Table 5.1: Effects of crude extract of *Aglaonema hookerianum (leaf)* on the Gastrointestinal Transit Test:

Serial no	Treatment	Dose (ml/kg or mg/kg)	Mean of % length of small intestine
1.	5% CMCmale (p.o)	10	52.50±8.4
2.	5% CMCfemale (p.o)	10	53.84±0.00
3.	Bisacodyl (p.o)	5	81.40±0.75
4.	Dose-1 male, (p.o)	400	55.96±2.96
5.	Dose-1 female(p.o)	400	48.33±1.67
6.	Dose-2 male, (p.o)	800	68.5840±2.93
7.	Dose-2 female(p.o)	800	61.43±1.89
8.	Dose2 (p.o)+Atropine (i.p)	800+10	58.93±4.23
9.	CMC(p.o)+Atropine (i.p)	10+10	46.5±5.1
10.	Dose2 Female(p.o)+Atropine (i.p)	800+10	56±0.00
11.	Dose2 Male(p.o)+Atropine (i.p)	800+10	58.93±4.23

Dose-1=400mg/kg (*Aglaonema hookerianum*)

Dose-2= 800 mg/kg (*Aglaonema hookerianum*)

Aglaonema hookerianum = Aglaonema hookerianum

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

All the test group of crude extract except group-2 showed significant increased transit ($p<0.001$, $p<0.01$ and $p<0.05$) of GI motility test at a dose of, 400mg/kg and 800 mg/kg. For each doses (Dose-1, 2) 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-2 showed highest percentage of the intestinal length traversed by the charcoal (68.5840±2.93, $p<0.001$). The reference drug bisacodyl showed the percentage of length 81.40±0.75 ($p<0.001$) 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 but Crude extract of dose2+Atropine(male and female) did not showed most prominent percentage of the intestinal length traversed by the charcoal, that is (56±0.00 and 58.93±4.23 $p<0.001$)

Gastrointestinal Transit Test:

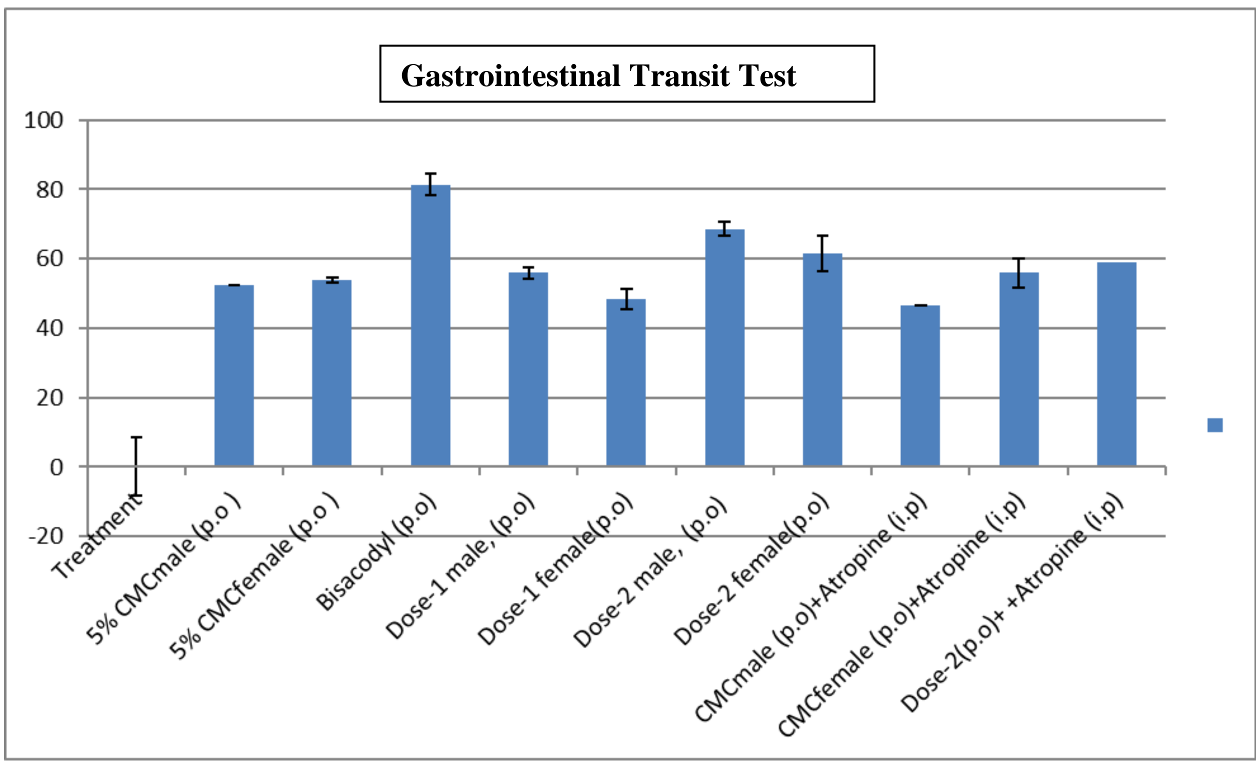

Figure 5.1: Bar diagram showing the dose-dependent effect of crude extract on the travel of charcoal meal through small intestine of mice, in the absence and presence of atropine. Each bar shown represents mean of 3 animals per group.

5.2. CNS Activity Test of Methanolic Extract of *Aglaonema hookerianum*

5.2.1. Open Field Test:

CNS of the methanolic extract of the bark part of the plant *Aglaonema hookerianum* 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 (Table5.2, 5.3 and 5.4 and Fig. 5.2, 5.3 and 5.4). The result was found to statistically significant. The experimental findings that are noted are below-

Table 5.2: CNS Activity of plant extract of *Aglaonema hookerianum* 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 *Aglaonema hookerianum*	200mg/kg	66.22±2.7	67.66±1.62	72.77±2.29	49.44±2.01	45.55±2.48
Crude extract of *Aglaonema hookerianum*	400mg/kg	76.44±2.25	60.22±2.83	65.22±5.14	52.33±2.30	43.00±2.00
Crude extract of *Aglaonema hookerianum*	800mg/kg	70.55±3.35	54.55±2.73	53.44±2.85	44.11±1.85	37.77±1.81
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

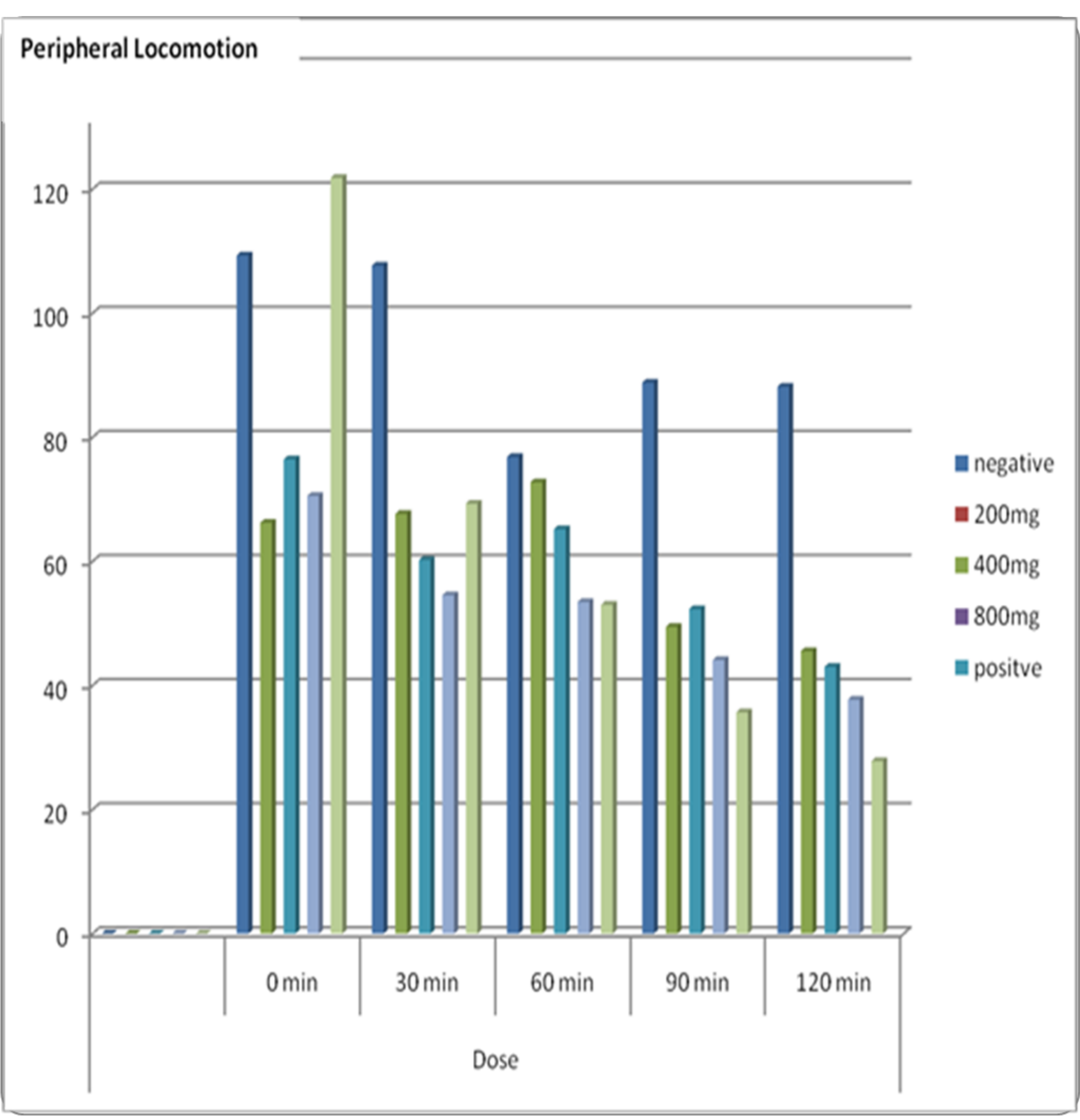

Figure 5.2: Graphical Presentation of CNS Activity of plant extract of *Aglaonema hookerianum* by Open Field Test (Peripheral Locomotion) in Mice.

Table 5.3: CNS Activity of plant extract of *Aglaonema hookerianum* 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 *Aglaonema hookerianum*	200mg/kg	2.44±0.44	3.00±0.41	2.55±0.50	3.00±0.47	1.22±0.32
Crude extract of *Aglaonema hookerianum*	400mg/kg	4.11±0.56	3.33±0.44	1.44±0.37	1.33±0.28	1.22±0.32
Crude extract of *Aglaonema hookerianum*	800mg/kg	5.22±0.57	3.55±0.55	2.33±0.33	2.77±0.40	1.77±0.46
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

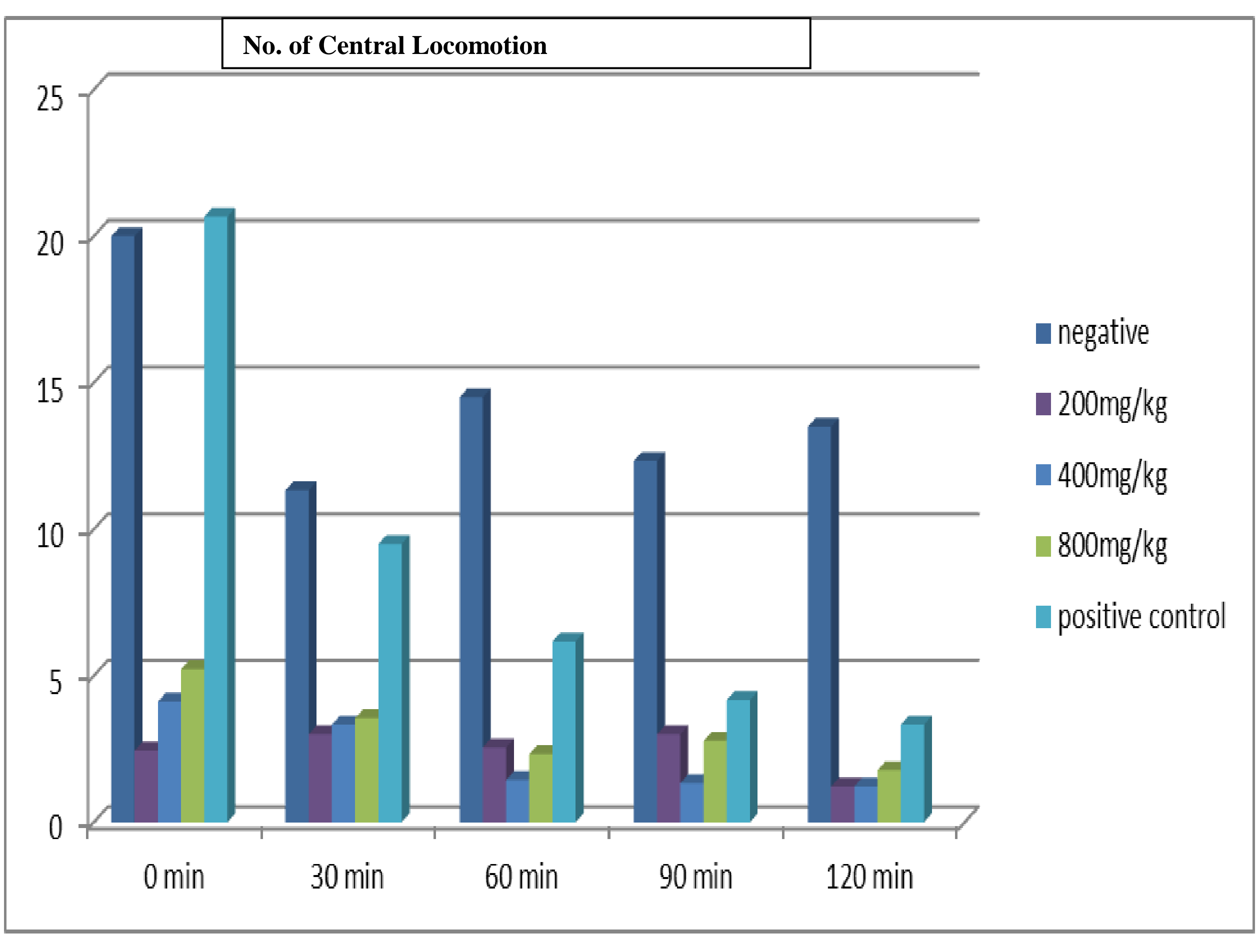

Figure 5.3: Graphical Presentation of CNS Activity of plant extract of *Aglaonema hookerianum* by Open Field Test (Central Locomotion) in Mice.

Table 5.4: CNS Activity of plant extract of *Aglaonema hookerianum* 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 *Aglaonema hookerianum*	200mg/kg	8.66±0.47	7.22±0.43	4.77±0.87	2.66±0.40	1.77±0.46
Crude extract of *Aglaonema hookerianum*	400mg/kg	12.66±0.40	9.33±0.57	2.66±0.40	1.55±0.33	0.77±0.27
Crude extract of *Aglaonema hookerianum*	800mg/kg	11.44±0.76	8.88±0.58	4.22±0.81	1.88±0.38	0.55±0.24
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

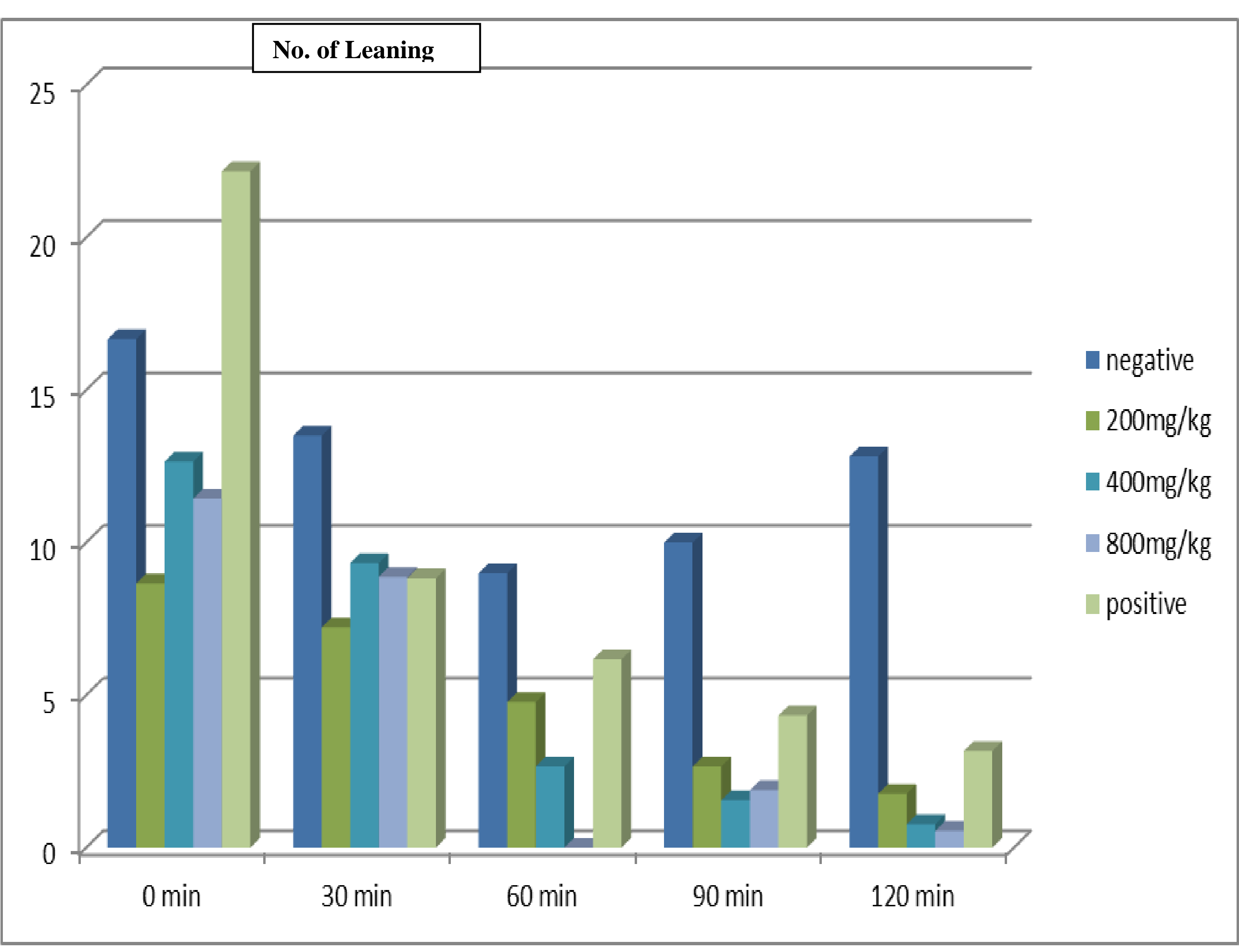

Figure 5.4: Graphical Presentation of CNS Activity of plant extract of *Aglaonema hookerianum* by Open Field Test (Leaning) in Mice.

Table 5.5: CNS Activity of plant extract of *Aglaonema hookerianum* by Open Field Test (Grooming) in Mice

Groups	Dose	No. of Grooming				
		0 min	**30 min**	**60 min**	**90 min**	**120 min**
Negative control 5% CMC	10ml/kg	0.50±0.22	0.50±0.22	0.50±0.22	0.67±0.22	0.46±0.21
Crude extract of *Aglaonema hookerianum*	200mg/kg	5.55±0.65	9.00±0.40	13.33±0.66	9.00±1.06	4.77±0.46
Crude extract of *Aglaonema hookerianum*	400mg/kg	2.44±0.29	12.77±1.13	15.00±1.22	10.77±0.46	6.77±0.57
Crude extract of *Aglaonema hookerianum*	800mg/kg	4.55±0.52	13.22±1.19	9.33±0.74	7.44±0.70	2.44±0.24
Positive control, Diazepam	1mg/kg	1.0±0.25	1.0±0.25	1.0±0.25	1.0±0.25	1.0±0.25

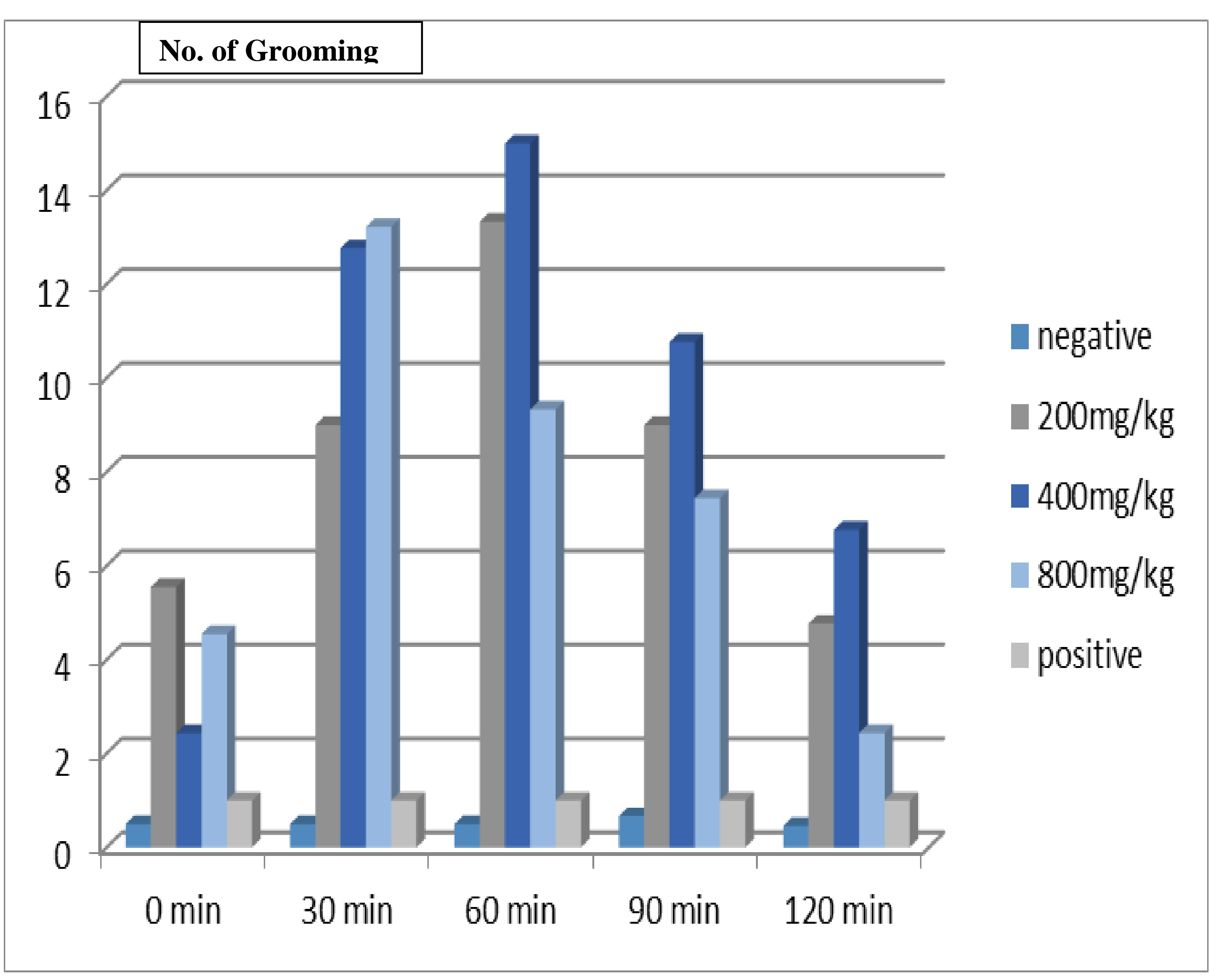

Figure 5.5: Graphical Presentation of CNS Activity of plant extract of *Aglaonema hookerianum* by Open Field Test (Grooming) in Mice.

Table 5.6 : Effect of methanolic extract of *Aglaonema hookerianum* 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
Aglaonema hookerianum **(200mg/kg)**	Male	18.84±0.62	36.06±1.06	3
Aglaonema hookerianum **(200mg/kg)**	Female	18.92±0.36	31.58±2.8	2
Aglaonema hookerianum **(400mg/kg)**	Male	19.94±0.84	36.50±0.50	2
Aglaonema hookerianum **(400mg/kg)**	Female	19.33±0.41	31.87±0.28	2
Aglaonema hookerianum **(800mg/kg)**	Male	19.72±0.69	41.62±0.37	2
Aglaonema hookerianum **(800mg/kg)**	Female	20.16±0.58	30.51±0.24	1

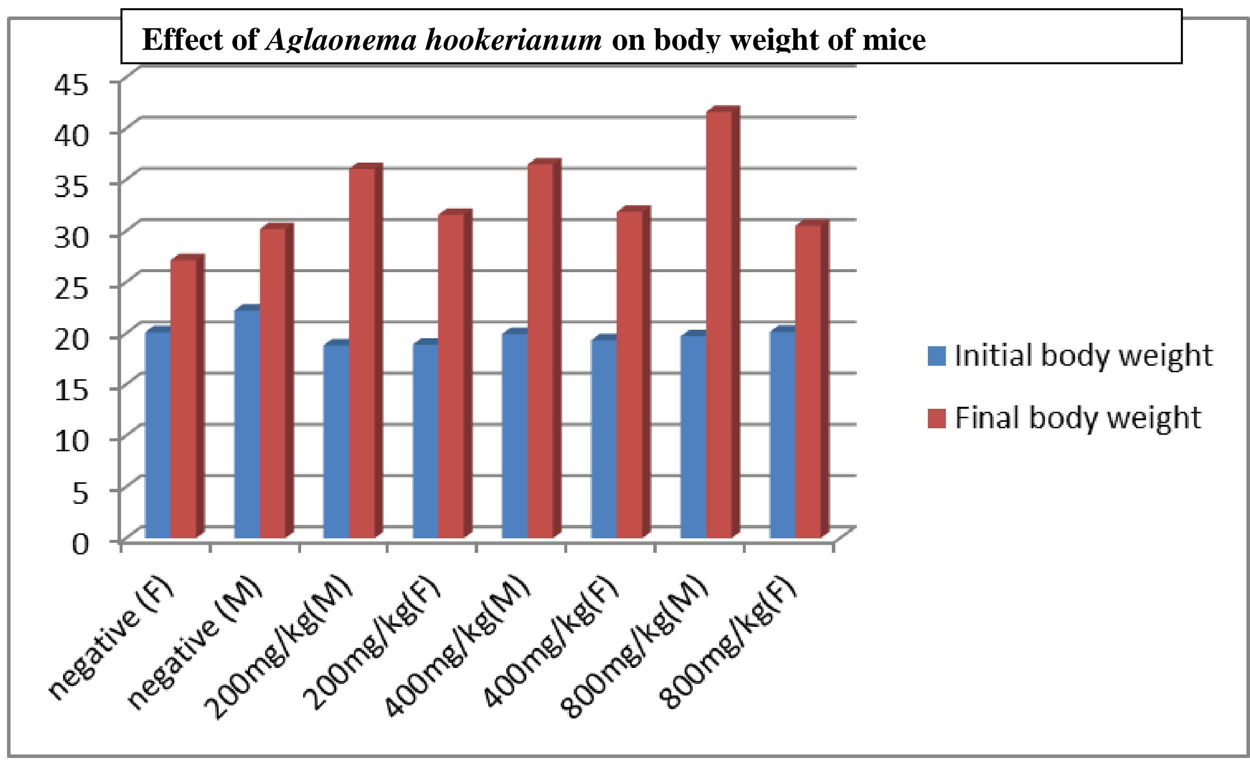

Figure 5.6: Graphical Presentation of Effect of methanolic extract of *Aglaonema hookerianum* on body weight in mice

5.7. Acute and Chronic Toxicity Test:

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

5.7.2. Chronic Toxicity Test:

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

Drug dose 200,400 and 800 mg/kg (CBC & Biochemical Test):

In the chronic study of methanolic extract of *Aglaonema hookerium* at a dose (200,400,800 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 *Aglaonema hookerianum* methanolic extract for a period of 90 days cannot induce significant aneamia. 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 *Aglaonema hookerianum.*

Table 5.7: Effect of *Aglaonema hookerianum* 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
Aglaonema hookerianum **(200mg/kg, female)**	6.97	14.6	78	3.7	-	3.7
Aglaonema hookerianum **(200mg/kg, male)**	21.91	-	-	2.7	0.1	-
Aglaonema hookerianum **(400mg/kg, female)**	11.3	12.4	-	7.1	0.6	-
Aglaonema hookerianum **(400mg/kg, male)**	5.33		69	2.9	-	7.4
Aglaonema hookerianum **(800mg/kg, female)**	8.78	12	82.7	4.4	-	1.4
Aglaonema hookerianum **(800mg/kg, male)**	13.94	-	-	4.5	-	-

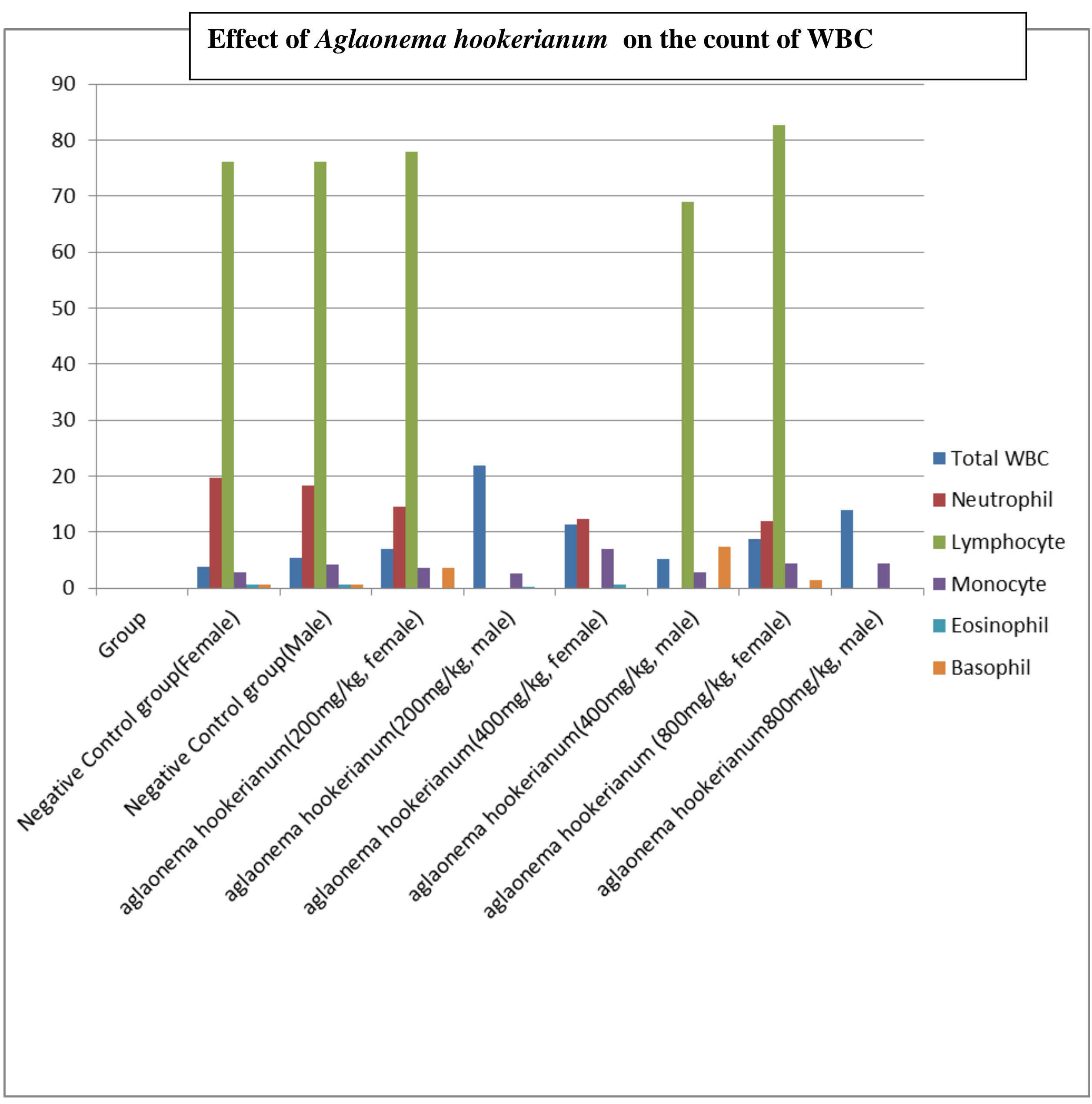

Figure 5.7 : Effect of *Aglaonema hookerianum* on the Different count of WBC (White Blood Cell)

Table 5.8 : Effect of *Aglaonema hookerianum* on the count of RBC (Red Blood Cell)

Treatment Group	Total RBC10^6/mm^3(n)	Haemoglobin	HCT	MCV	MCH	MCHC
Negative Control group(Female)	8.33	14.5	55.5	64.25	16.62	25.9
Negative Control group(Male)	8.79	13.55	50.3	56.7	15.28	26.93
Aglaonema hookerianum **(200mg/kg, male)**	7.06	8	30.4	43.1	11.3	26.3
Aglaonema hookerianum **(200mg/kg, female)**	8.56	11	41.2	48.1	15	30.1
Aglaonema hookerianum **(400mg/kg, male)**	8.68	12.7	40.3	48.4	15.2	31.5
Aglaonema hookerianum **(400mg/kg, female)**	8.33	13.2	49.5	48.5	14.6	30
Aglaonema hookerianum **(800mg/kg, Male)**	8.8	12.8	37.7	42.8	14.5	34
Aglaonema hookerianum **800mg/kg, Female)**	8.69	12.9	38	43.7	15.9	31.4

Effect of *Aglaonema hookerianum* on the count of RBC

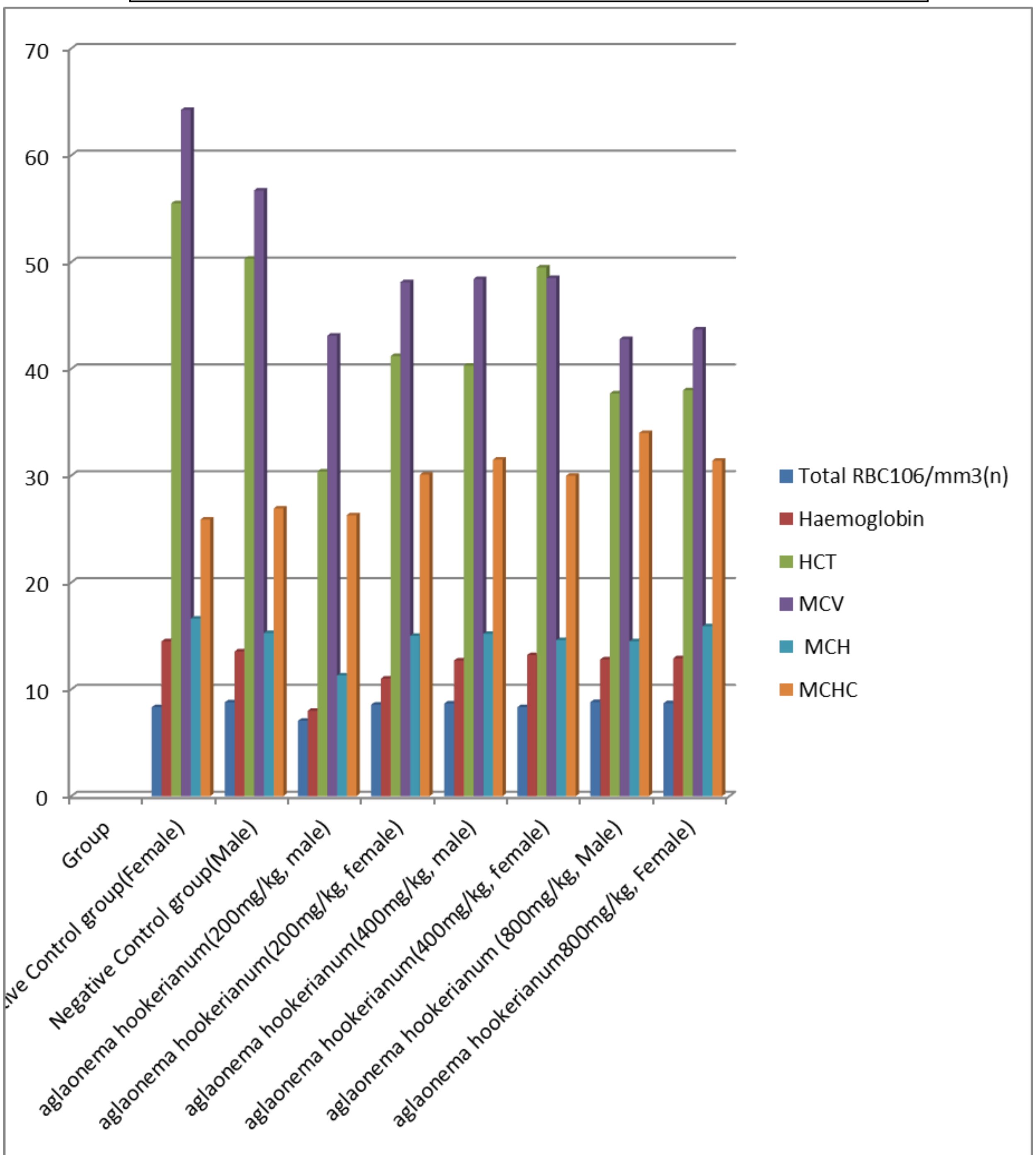

Figure 5.8: Effect of *Aglaonema hookerianum* on the count of RBC (Red Blood Cell)

Table 5.9: Effect of *Aglaonema hookerianum* on Platelet count on the CBC (Count Blood Cell) Test

Treatment Group	Platelet $10^3/mm^3$(n)
Negative Control group(Female)	848
Negative Control group(Male)	1211
***Aglaonema hookerianum*(200mg/kg, male)**	1622
***Aglaonema hookerianum*(200mg/kg, female)**	778
***Aglaonema hookerianum*(400mg/kg, male)**	1685
***Aglaonema hookerianum*(400mg/kg, female)**	985
***Aglaonema hookerianum* (800mg/kg, Male)**	1195
***Aglaonema hookerianum*800mg/kg, Female)**	1074

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

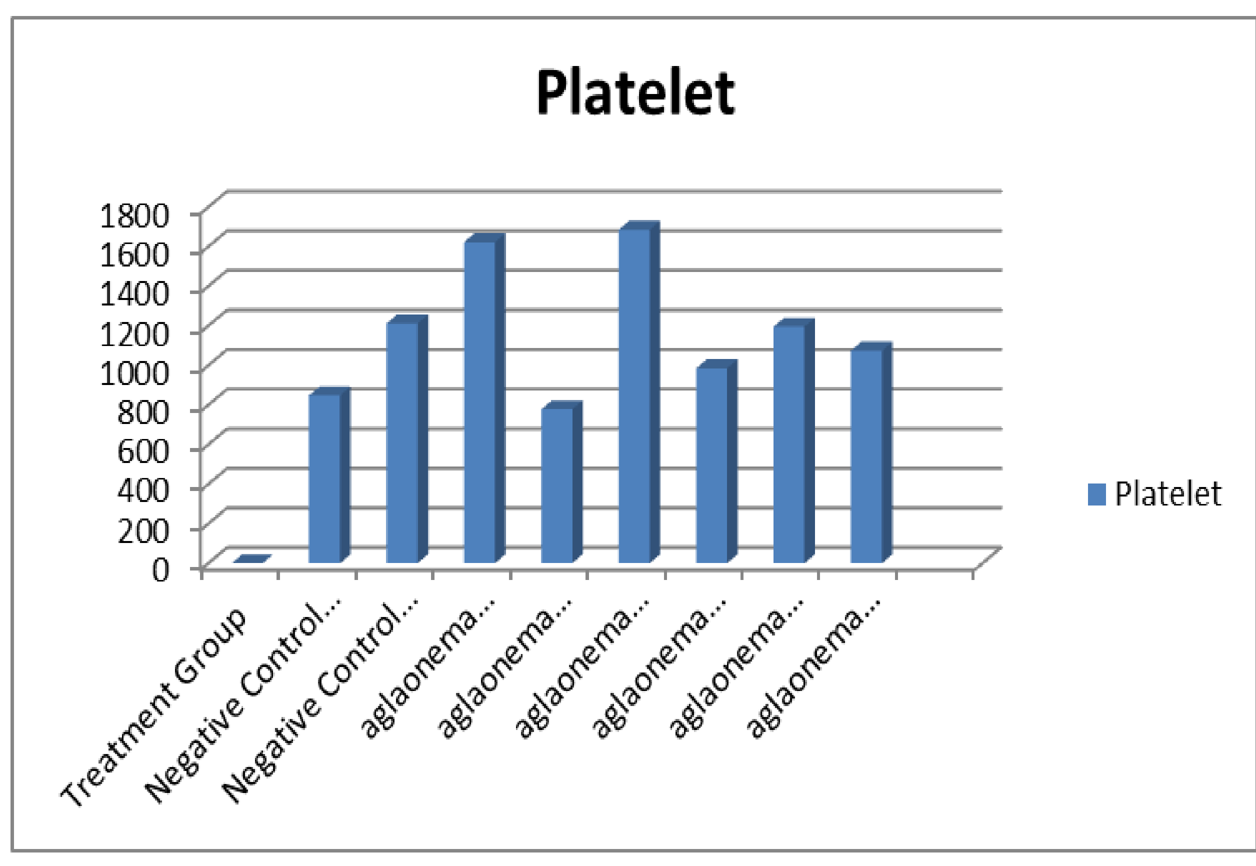

Figure 5.9: Effect of *Aglaonema hookerianum* on Platelet on the CBC (Count Blood Cell) Test

Table 5.10: Effect of *Aglaonema hookerianum* on the Liver Function Test

Treatment Group	SGPT (IU/dl)	SGOT (IU/dl)	ALT (IU/dl)
Negative Control group(Female)	55.83	33.33	102.17
Negative Control group(Male)	49.17	31.83	341.5
*Aglaonema hookerianum(*200mg/kg, male)	63	12	90
*Aglaonema hookerianum(*200mg/kg, female)	76	6	67
*Aglaonema hookerianum(*400mg/kg, male)	95	32	114
*Aglaonema hookerianum(*400mg/kg, female)	32	47	64
Aglaonema hookerianum (800mg/kg, Male)	95	12	105
*Aglaonema hookerianum*800mg/kg, Female)	24	15	201

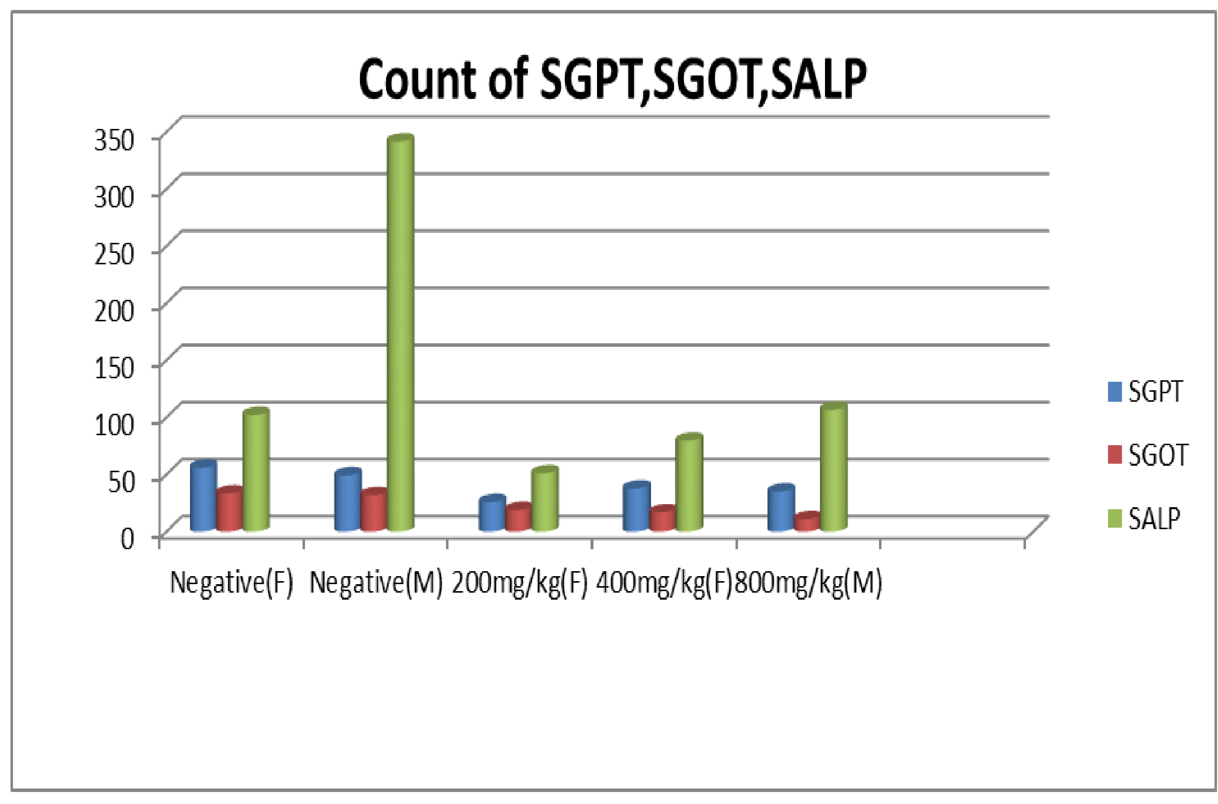

Figure 5.10: Effect of *Aglaonema hookerianum* on the Liver Function Test

5.11. Result and discussion of the thrombolytic activity of *Aglaonema hookerianum* :

As a part of discovery of cardio protective drugs from natural source ,the extract of ***Aglaonema hookerianum*** assessed for thrombolytic activity and the result are predicted in the table of 100 micro litre,a positive control to the clot and subsequent incubation for 90 minutes at 37 degree c.showed highest 41.75% which is the methanolic exract of Aglaonema hookerium(Leaf) and for bark it show the highest clot lysis of 24.27%.

leaf					
Dose	Blood source	Code	Clot formation	clot	Clot lysis
1mg/ml	Person 1	A1l			
	Person 2	A2l			
5mg/ml	Person 1	A3l			
	Person 2	A4l			
10mg/ml	Person 1	A5l			
	Person 2	A6l			

Fig**5.11(a)**: clot lysis of ***Aglaonema hookerianum*** (Leaf)

bark					
Dose	Blood source	Code	Clot formation	clot	Clot lysis
1mg/ml	Person 1	A1l			
	Person 2	A2l			
5mg/ml	Person 1	A3l			
	Person 2	A4l			
10mg/ml	Person 1	A5l			
	Person 2	A6l			

Fig **5.11(b)**: Clot lysis of ***Aglaonema hookerianum*** (Bark)

Table **5.11: Comparison between *Aglaonema hookerianum* leaf and bark on thrombolytic test result:**

Dose	**Blood source**	***Aglaonema hookerianum* LEAF**	***Aglaonema hookerianum* BARK**	**NEGATIVE**	**POSITIVE**
1mg/ml	Person 1	24.69399	10.02629	6.896552	52.2523
	Person 2	39.80293	16.93638	8.057203	50.2075
5mg/ml	Person 1	41.75824	24.08648	8.432148	56.5401
	Person 2	15.78298	9.340486	8.15508	49.8024
10mg/ml	Person 1	21.08234	13.93963	12.40033	52.5424
	Person 2	24.27912	12.04044	11.77876	56.993

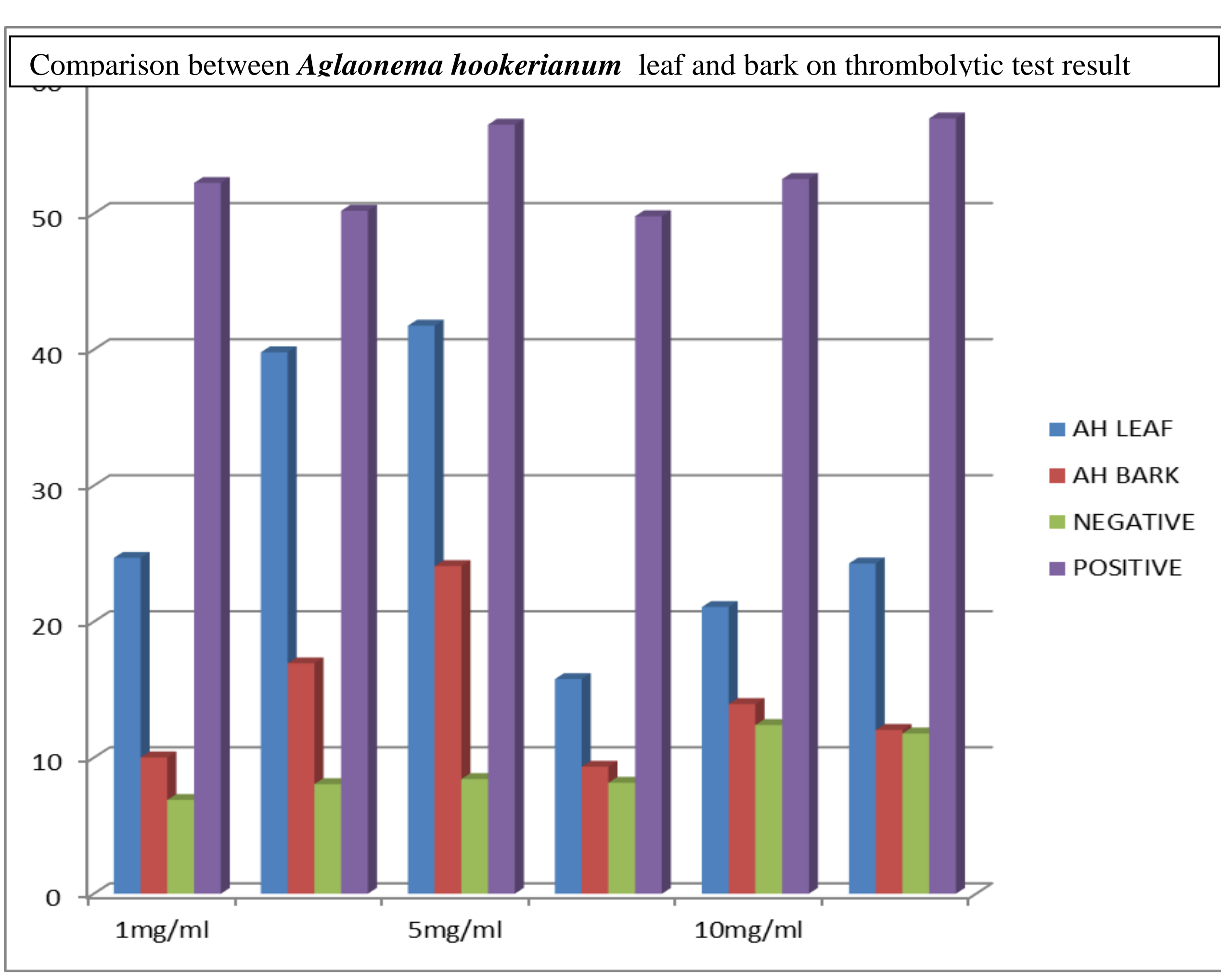

Fig**5.12**: Comparison between ***Aglaonema hookerianum*** leaf and bark on thrombolytic test result

6.Antioxidant:

6.1 Total Phenolic Content Assay

The Methanolic extract of ***Aglaonema hookerianum*** *fraction* of methanolic extract were subjected to Total Phenolic Content Assay by the method described by *(Arpona et al., 2013)*. Here, Total Phenolic Content was measured as Salicylic acid equivalence.

Table 6.1.1 : Standard Curve Preparation by Using Salicylic Acid

Standard Curve Preparation by Using Salicylic Acid

SL	Concentration mg/ml	Absorbance	Regression Equation	R^2
1	0.625	0.433	Y = 0.109x + 0.418	0.983
2	1.25	0.528		
3	2.5	0.768		
4	5	0.998		
5	10	1.480		

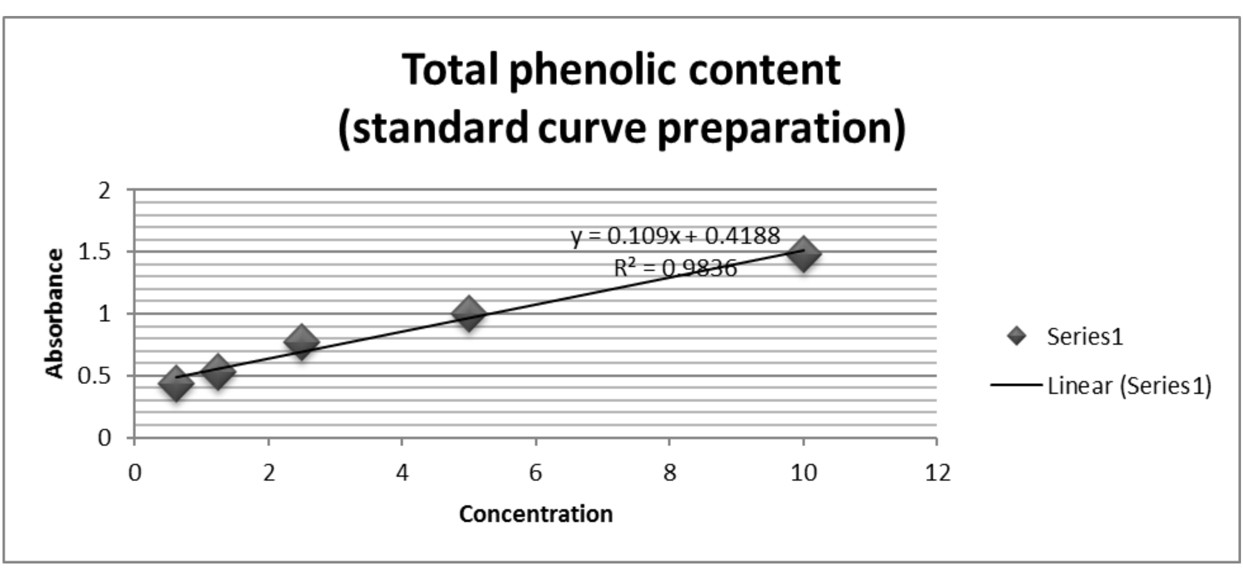

Fig 6.1.1.: Total Phenolic Content (Standard Curve)

Table 6.1.2: Total Phenolic Content of *Aglaonema hookerianum*

Absorbance	**mgSAE/g**	**Mean**
1.751	122.2	122.6
1.757	122.8	
1.759	123.02	

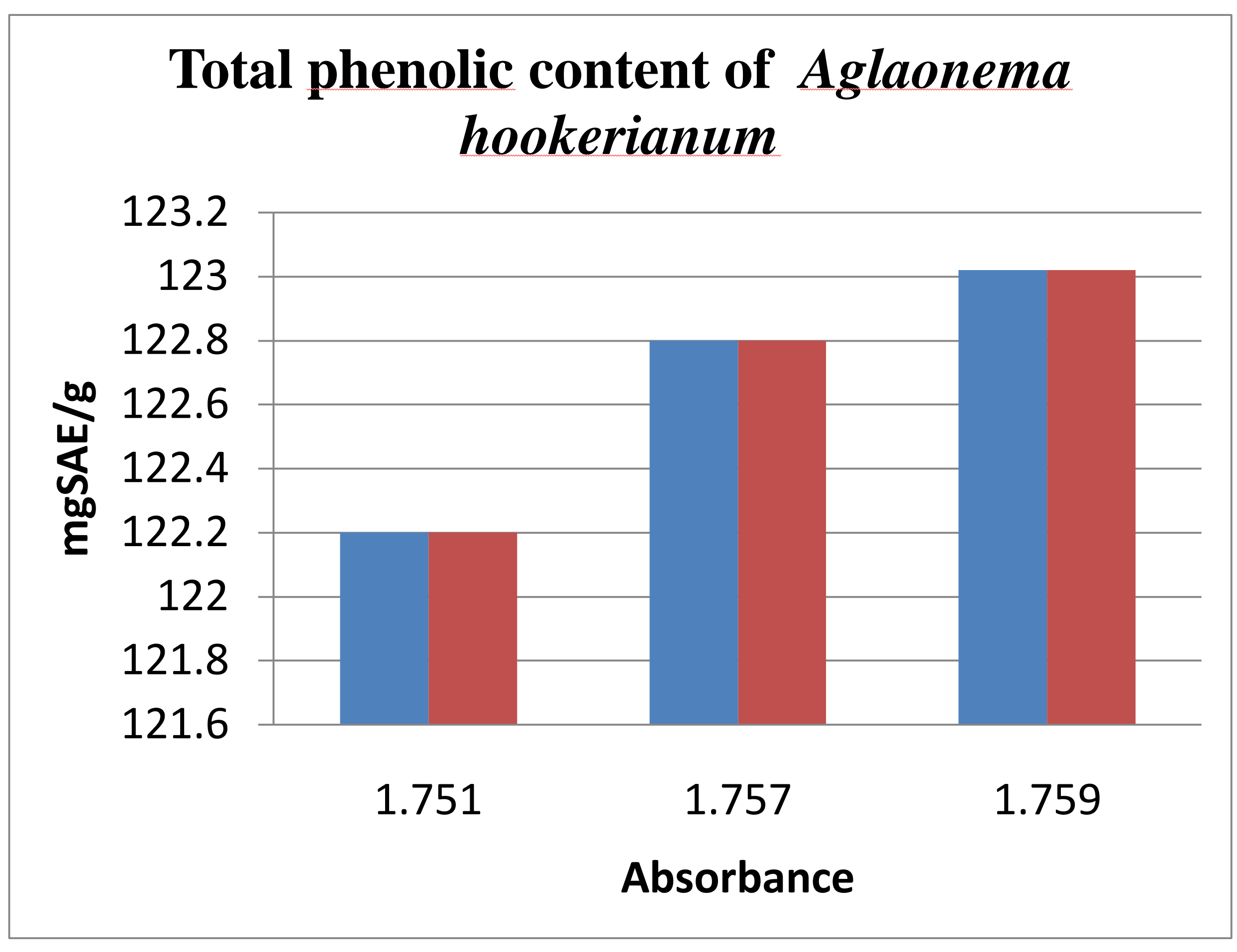

Fig6.1.2: Total Phenolic Content of *Aglaonema hookerianum*

Discussion: From the above result we can see that methanolic extract of *Aglaonema hookerianum* (122.6 mgSAE/g)

Evaluation of Antioxidant Activity

6.2. DPPH Radical Scavenging Assay

The methanolic extract of *Aglaonema hookerium* of methanolic extract of *Aglaonema hookerianum* were subjected to DPPH Radical Scavenging Assay according to method described by (*Arpona et al., 2013)* and ascorbic acid was used as reference standard in this experiment.

Table 6.2.1: DPPH Radical Scavenging Assay of Ascorbic Acid (Standard)

Absorbance of Blank	Concentration µg/ml	Log Concentration	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.625	0.98	- 0.009	0.536	14.24	4.35
	1.95	0.290	0.508	18.72	
	3.91	0.592	0.365	41.60	
	7.81	0.893	0.198	68.32	
	15.63	1.194	0.098	84.32	
	31.25	1.495	0.058	90.72	
	62.5	1.796	0.042	93.25	
	125	2.097	0.027	95.68	
	250	2.398	0.021	96.32	
	500	2.699	0.015	97.60	

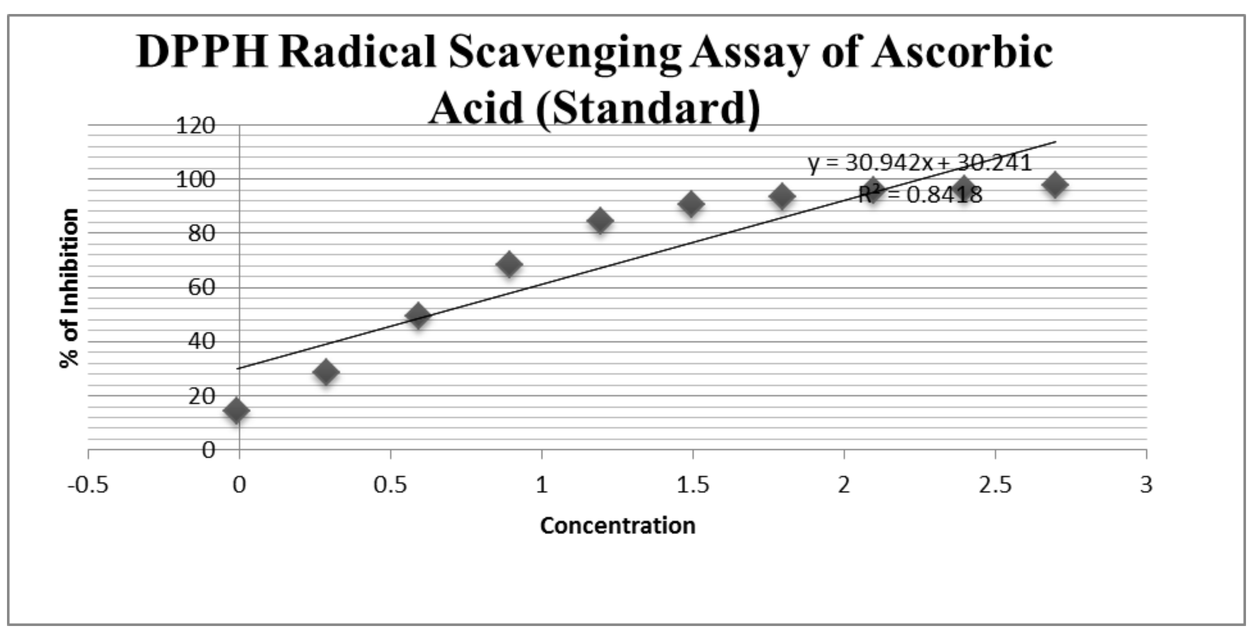

Fig 6.2.1: DPPH Radical Scavenging Assay of Ascorbic Acid (Standard)

Table 6.2.2: DPPH Radical Scavenging Assay of *Aglaonema hookerianum*

Absorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.459	0.98	0.457	0.44	11.08
	1.95	0.452	1.52	
	3.91	0.445	3.05	
	7.81	0.438	4.57	
	15.63	0.428	7.40	
	31.25	0.405	11.76	
	62.5	0.400	12.85	
	125	0.358	22	
	250	0.246	46.41	
	500	0.101	77.99	

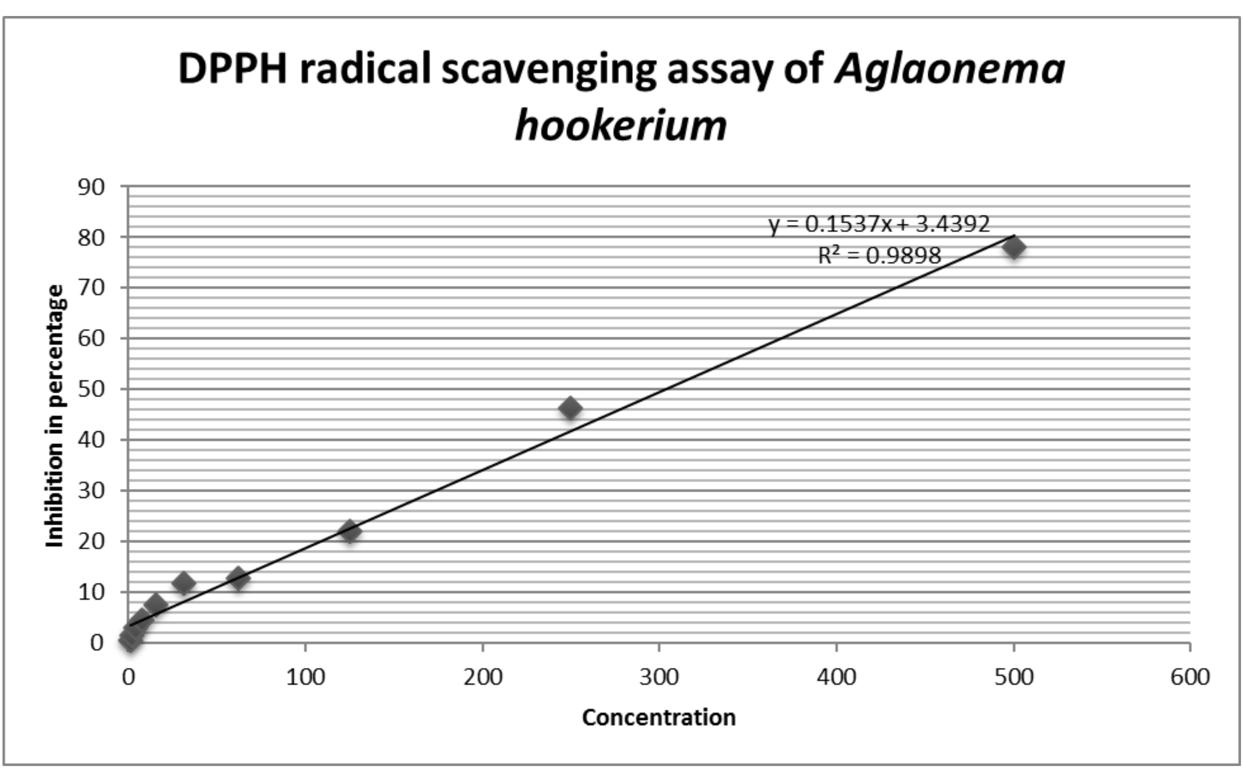

Fig 6.2.2: DPPH Radical Scavenging Assay of *Aglaonema hookerianum.*

Discussion: The result of the tests is present in the following figure. The extract demonstrated an antioxidant activity by using DPPH Where DPPH radical activity of methanolic extract of *Aglaonema hookerium* is good (IC_{50} 11.08 µg/ml)

6.4. Reducing Power Assay

The methanolic extract of *Aglaonema hookerianum* were subjected to Reducing Power Assay according to method described by (*Arpona et al., 2013*) here, ascorbic acid was used as reference standard.

Table 6.3.1: Reducing Power Assay of Ascorbic acid (Standard)

SL.	Concentration µg/ml	Absorbance			Mean
		1	2	3	
1	0.98	0.099	0.098	0.097	0.098
2	1.95	0.101	0.105	0.103	0.103
3	3.91	0.143	0.145	0.146	0.144
4	7.81	0.221	0.222	0.221	0.221
5	15.63	0.283	0.284	0.285	0.284
6	31.25	0.486	0.488	0.489	0.487
7	62.5	0.952	0.955	0.953	0.953
8	125	1.434	1.435	1.434	1.434
9	250	1.978	1.978	1.976	1.977
10	500	2.567	2.567	2.566	2.566

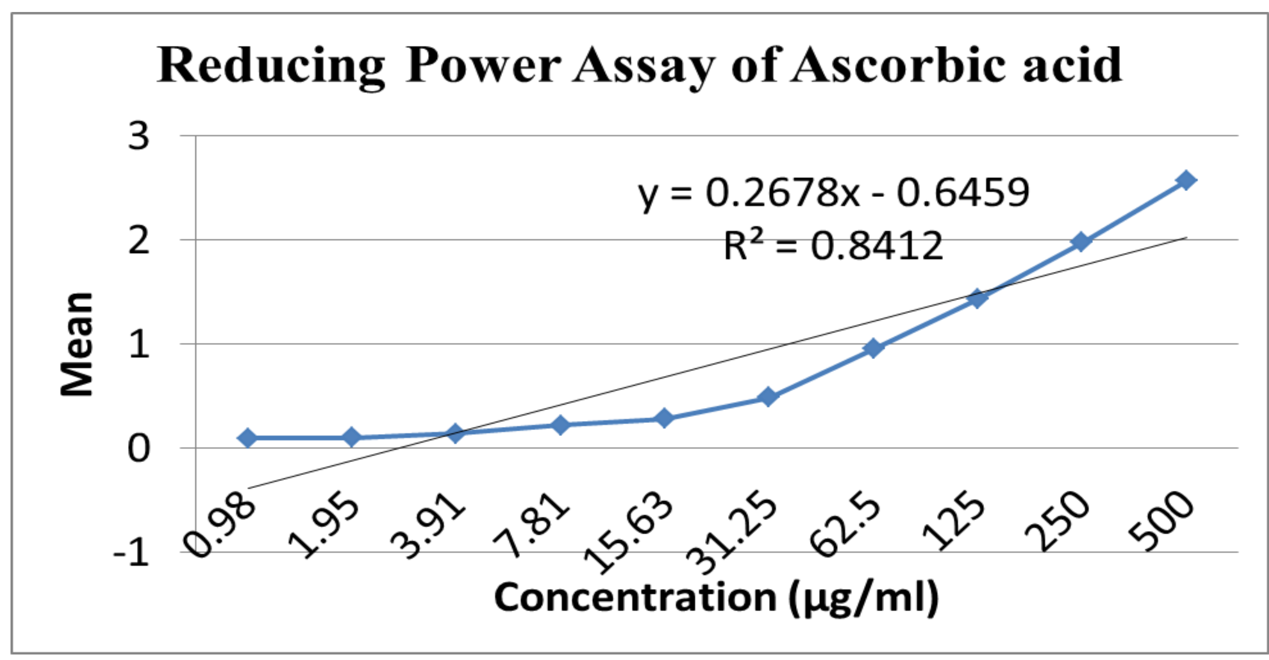

Fig6.4.1: Reducing Power Assay of Ascorbic acid

Table 6.3.2.: Reducing Power Assay of *Aglaonema hookerianum*

SL.	Concentration µg/ml	Absorbance			Mean
		1	2	3	
1	0.98	0.034	0.033	0.035	0.034
2	1.95	0.047	0.046	0.048	0.047
3	3.91	0.059	0.057	0.060	0.058
4	7.81	0.078	0.078	0.077	0.077
5	15.63	0.099	0.098	0.101	0.099
6	31.25	0.128	0.127	0.128	0.127
7	62.5	0.173	0.171	0.173	0.172
8	125	0.213	0.212	0.214	0.213
9	250	0.343	0.341	0.342	0.342
10	500	0.497	0.496	0.499	0.497

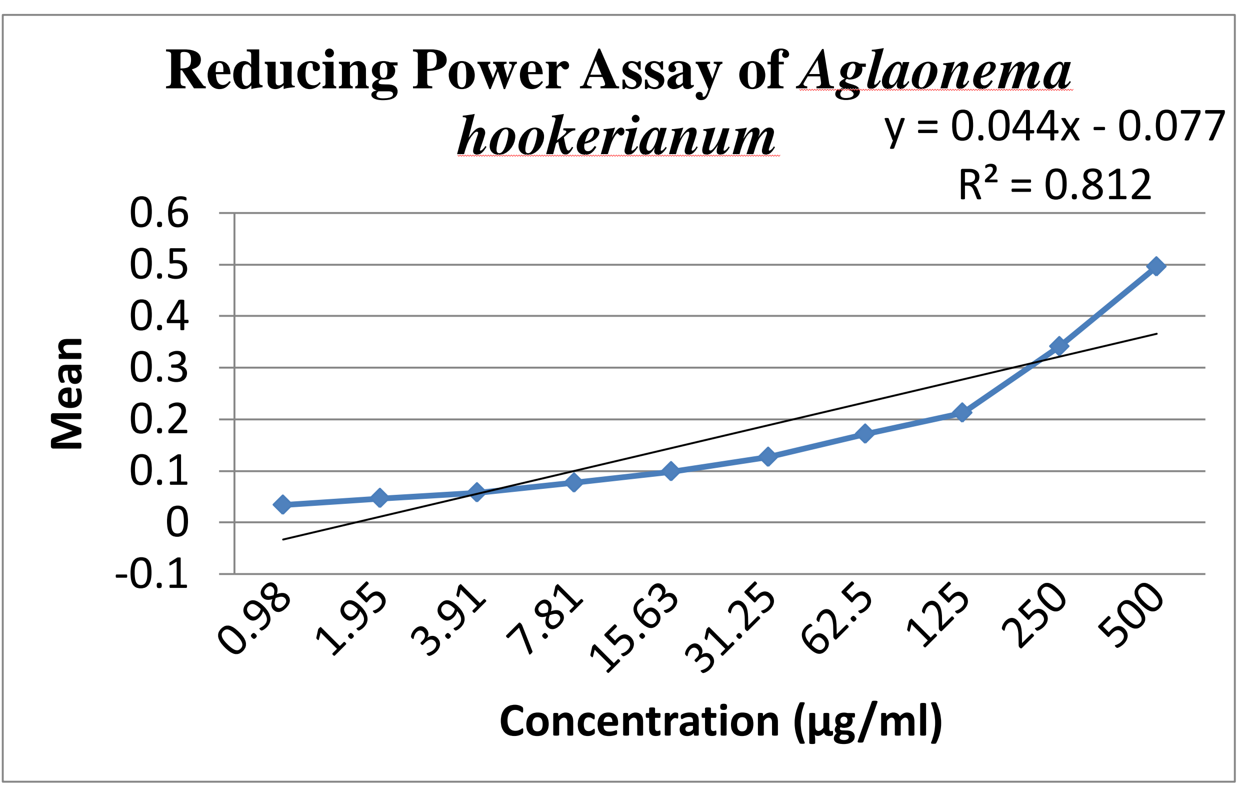

Fig **6.3.2**: Reducing Power Assay of *Aglaonema hookerianum*

Discussion:

In reducing power assay higher absorbance of the reaction mixture indicates higher reductive potential. In the graph above we can see that with the increase of concentration absorbance is also increase.

The reducing capability of a compound may serve as a significant indicator of its potential antioxidant activity. Further studies will help in identifying the individual compounds that aids in the reducing power and to identify the synergistic effect.

Chapter 06

Conclusion

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 unparallel 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, Thrombolytic activities, Antioxidant activity, Acute and Chronic toxicity of methanolic extract of *Aglaonema hookerianum* leaf.

This study shows that the prokinetic and laxative activities of extracts of *Aglaonema hookerium (*leaf) in mice are partially mediated through muscarinic receptors. Thus, this study provides sound mechanistic basis for the medicinal use of *Aglaonema hookerianum(*leaf) 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 *Aglaonema hookerianum (*Leaf) (200mg/kg, 400mg/kg & 800mg/kg) dose dependently reduces the number of peripheral locomotion, central locomotion, leaning and grooming 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. 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.

In the methanolic extract of *Aglaonema hookerianum* (leaf) it showed highest 41.75% thrombolytic activity and moderate activity showed by the above experiment compared with the streptokinase and *Aglaonema hookerianum (* Bark).

In total phenolic content assay of methanolic extract of *Aglaonema hookerianum* have the phenolic content of (122.6 mgSAE/g). In DPPH radical activity of methanolic extract of

Aglaonema hookerianum is good (IC_{50} 11.0(µg/ml). In reducing power assay higher absorbance of the reaction mixture indicates higher reductive potential. In this assay, *Aglaonema hookerianum* shows as the concentration increases the absorbance is also increases .

The aim of the study was also to investigate the possible toxicity of the plant *Aglaonema hookerianum* 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 test and histopathological Studies. CBC test and hepatic enzyme test are done by hematological machine and histopathological studies by microscopic test. The results of several widely accepted protocols would suggest that there were positive modulations in all the parameters of study in the *Aglaonema hookerianum* 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 *Aglaonema hookerianum* is safe in mice that is no significant change with dose when compare with negative control. The histopathological status of the liver tissues of both the treated mice where normal cellular architecture with prominent central vain was shown which indicates that the extract cannot cause damage to liver if used for maximum dose. This becomes important because liver is the primary organ for detoxification.

From the present investigation, it can be concluded that the methanolic extract of *Aglaonema hookerianum* (leaf) exhibited Laxative activity, CNS activity, Thrombolytic activity, Antioxidant activity and shows toxicity safety in acute and chronic toxicity studies in mice.

Chapter 07

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