Study of Pharmacological and toxicological Activities of Methanolic Extract of *Grewia asiatica*

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

Declaration by the Candidate

I, **Sabrina Islam (ID No. 2013-3-70-062)** Hereby declare that the dissertation entitled ***"*Study of Pharmacological and toxicological Activities of Methanolic Extract of *Grewia asiatica*"** 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.

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Certificate by the Supervisor

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DEDICAITON

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

ABSTRACT

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

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

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

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

The aim of the study was also to investigate the possible toxicity of the plant and especially to esta *Grewia asiatica* blish 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 *Grewia asiatica* may have several pharmacological activities but to prove the hypothesis it need further higher studies of *Grewia asiatica*

Keywords: *Grewia asiatica*, Laxative effect, Neuropharmacological effect, antioxidant effect, thrombolytic effect and Toxicity test.

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Chapter 01
Introduction

1.Introduction

1.1. Medicinal Plant

The medicinal use of plants is probably as old as mankind itself. Plants have continued to be a valuable source of natural products for maintaining human health, as studies on natural therapies have intensified. More than 150,000 plant species have been studied, and several of them contain therapeutic substances. The use of plant compounds for pharmaceutical purposes has gradually increased. According to the World Health Organization medicinal plants are probably the best source of a variety of drugs. About 80 % of individuals in developed countries use traditional medicine containing compounds derived from medicinal plants (Varalakshmi, et al. 2011).

Medicinal plants, defined as plants used for maintaining health and/or treating specific ailments, are used in a plethora of ways in both allopathic and traditional systems of medicine in countries across the world. Even people using only allopathic medicine throughout their lives are likely to be somewhat medicinal plant reliant as 20-25% of drugs prescribed are plant derived (Hall, et al. 2012).

1.1.1. Definitions of medicinal plants

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

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

1.1.2 Importance of Medicinal Plant

Plants are the tremendous source for the discovery of new products with medicinal importance in drug development. Today several distinct chemicals derived from plants are important drugs, which are currently used in one or more countries in the world. Herbal medicines have been utilized for many purposes, particularly in medical care as antiasthmatics (86.79 %), anti-rheumatics (62 %), diuretics (60.22 %), antiinflammation (29.62 %), anticancer (9.75 %), antidiabetics (8.33 %), antimicrobials, antifungals, antioxidants, antiallergy, analgesics, anti-obesity and antihypertention. In dental care it has been employed as anticariogenic, analgesic, local anesthetic, wound healing agents, anti-inflammation and recurrent aphthous stomatitis treatment etc. (Ghani, , et al. 2003).

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

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

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

1.1.3 Medicinal plants & Traditional Medicine Practice in Bangladesh

The plants which are useful for healing several diseases are called medicinal plant. There are 722 medicinal plants in our country. Bangladesh possesses a rich flora of medicinal plants. Out of the estimated 5000 species of different plants growing in this country more than a thousand are regarded as having medicinal properties. Out of them, more than a thousand have been claimed to possess medicinal 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 diarrheal diseases and 47 for diabetes (Ghani,, et al. 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 neighborhood.

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, molded 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” and, generally, the extensive traditional use of herbal products is not assumed to be based on a comprehensive well documented logic, but rather on empirical wisdom accumulated over many years, often arrived at through trial and error and transmitted orally from generation to generation. This traditional methodology has enabled those herbal medicines producing acute and obvious signs of toxicity to be well recognized and their use

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

1.1.5. Causes of toxicity with herbal products

All chemicals may be considered toxic under certain conditions, e.g. even pure water when inhaled is rapidly absorbed across the lung alveoli to cause lysis of red blood cells. But some chemicals present a greater hazard than others (Pascoe, 1983). A large number of plants contain appreciable levels of biosynthetically produced chemical substances and many of these have either been reported to be toxic to humans or are predictably toxic based on extensive animal or *in vitro* studies (Tomlinson and Akerele ,, et al. 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,, et al. 1999).

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, et al. 2000).

• The second category of causes of toxicity of herbal medicines is more extrinsic or non-associated with the plant active constituents. In this category, the toxicity is a result of exposure to plant products contaminated with excessive or banned pesticides, microbial contaminants, heavy metals or chemical toxins, or with substituted ingredients. The pesticide,

heavy metal and microbial contaminants may be linked to the source, collection or processing of the herbal materials (e.g.in contaminated environments).

1.1.6. Prevalence of toxicity with herbal products

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

From published reports, it appears that side effects or toxic reactions, of any form but associated with herbal medicines, are rare, 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 (Gaillard and Pepin, , et al. 1999).

1.2. Constipation

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

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

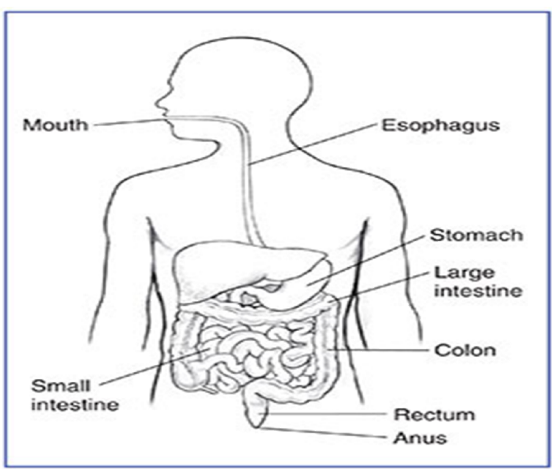


Figure 1.1: Human Gastrointestinal Tract (Higgins et al.2004)

1.2.1 Symptoms of constipation can include:

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

1.2.2. Causes of Constipation

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

Common factors or disorders that lead to constipation are

- Inadequate water intake
- Inadequate fiber in the diet
- A disruption of regular diet or routine; traveling

- Inadequate activity or exercise or immobility
- Eating large amounts of dairy products
- Stress
- Resisting the urge to have a bowel movement, which is sometimes the result of pain from hemorrhoids
- Hypothyroidism
- Neurological conditions such as Parkinson's disease or multiple sclerosis
- Depression
- Eating disorders
- Irritable bowel syndrome
- Pregnancy

Colon cancer (Higgins et al.2004)

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. (Higgins et al.2004)

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. (Higgins et al.2004)

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. (Higgins et al.2004)

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

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. (Higgins et al.2004)

1.2.6. Functional GI Disorders

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

1.2.7. Constipation Complications

For those people who suffer from chronic or severe constipation, complications can occur. The complications of constipation range from nagging to serious. A visit to the doctor for a diagnosis and treatment is the best way to avoid the complications of constipation.(Longstreth et al. 2006).

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(. Longstreth et al.2006).

1.2.9. The Complication of Fecal Impaction

A fecal impaction is the accumulated and hardened stool in the large intestine as a result of constipation. The symptoms of a fecal impaction include no solid stool being passed and liquid stool leaking from the colon. Only liquid can pass, because the colon is impacted and blocked. A fecal impaction can be a serious complication, especially if it causes an intestinal blockage and remains untreated.

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

1.2.10. Diagnosis of Constipation

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

1.2.11. Diagnostic Tests

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

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

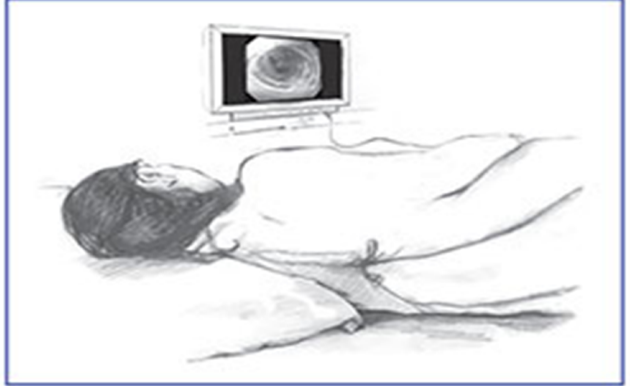


Figure 1.2: Flexible sigmoidoscopy (. Longstreth et al.2006).

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)

1.1.4. Nervous System

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.

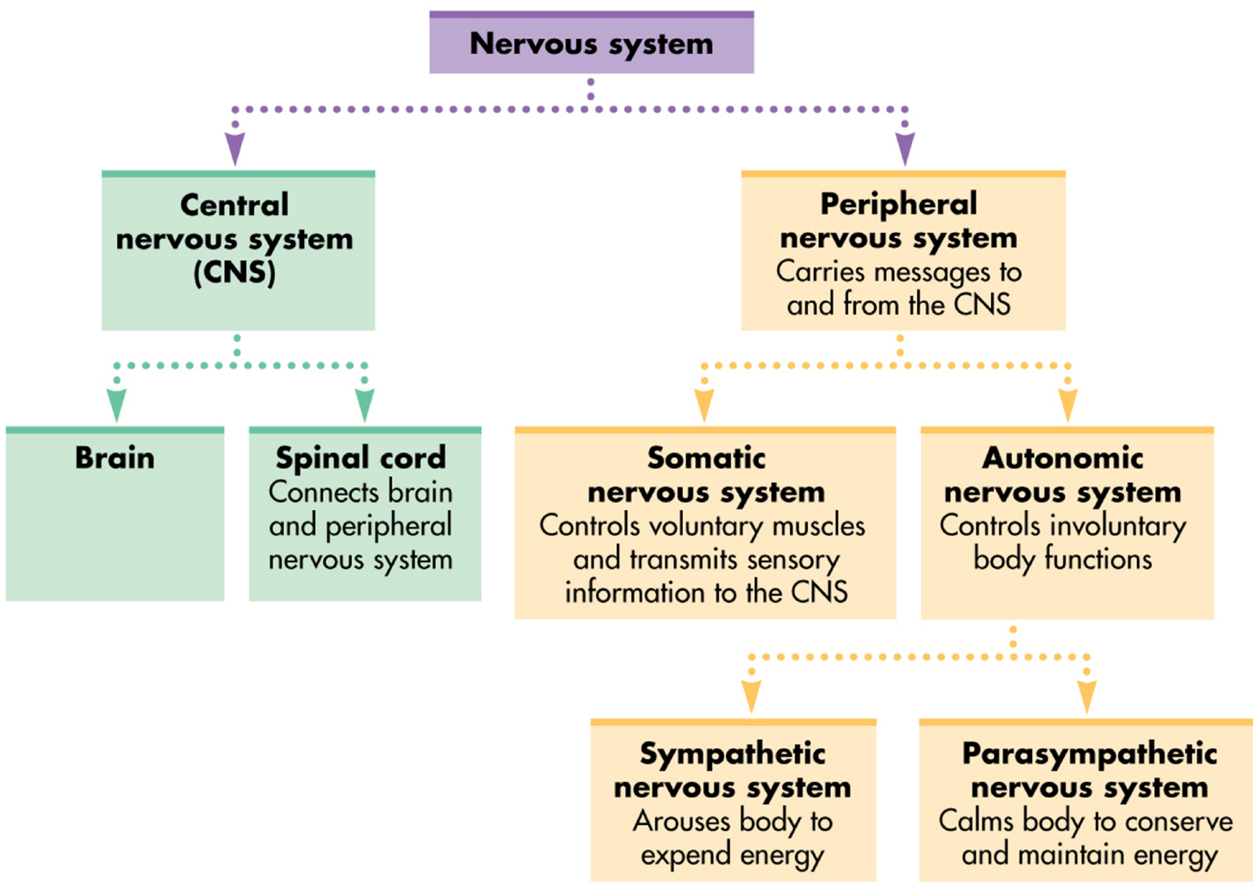


Figure1.3: Organization of the Human Nervous System. (Longstreth et al. 2006).

1.3.1. Thecentral 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)

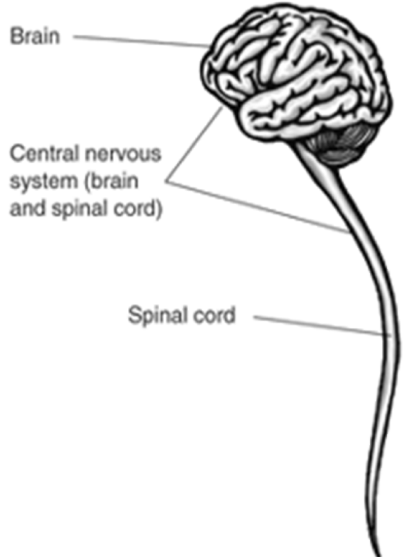


Figure 1.4: Central Nervous System (Longstreth et al.2006).

1.3.1.1. Parts of Central Nervous System

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

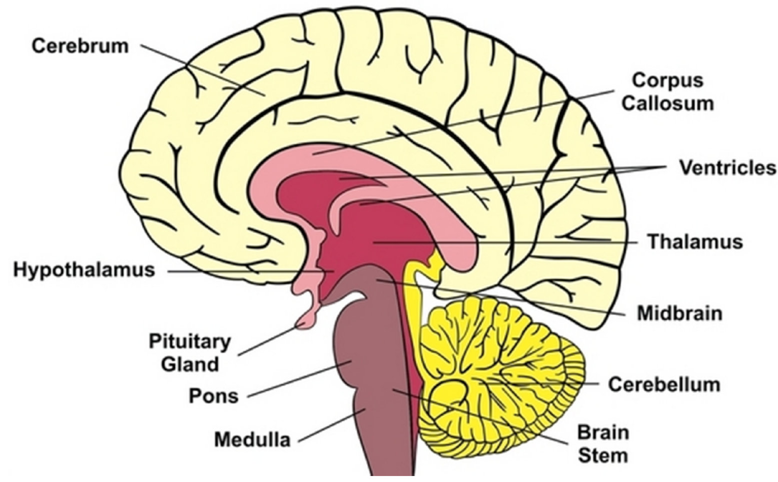


Figure : 1.5 Parts of CNS (Kandel, et al. 2000)

send information fromthe CNS to the muscles and glands.The peripheral nervous system isfurther divided into the somatic systemand 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 oftenwork in opposition to each other toregulate the involuntary processes ofthe body. Involuntary processes, such as heartbeat and peristalsis, are those that donot require or involve conscious control.

1.3.3. Nerve cells

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

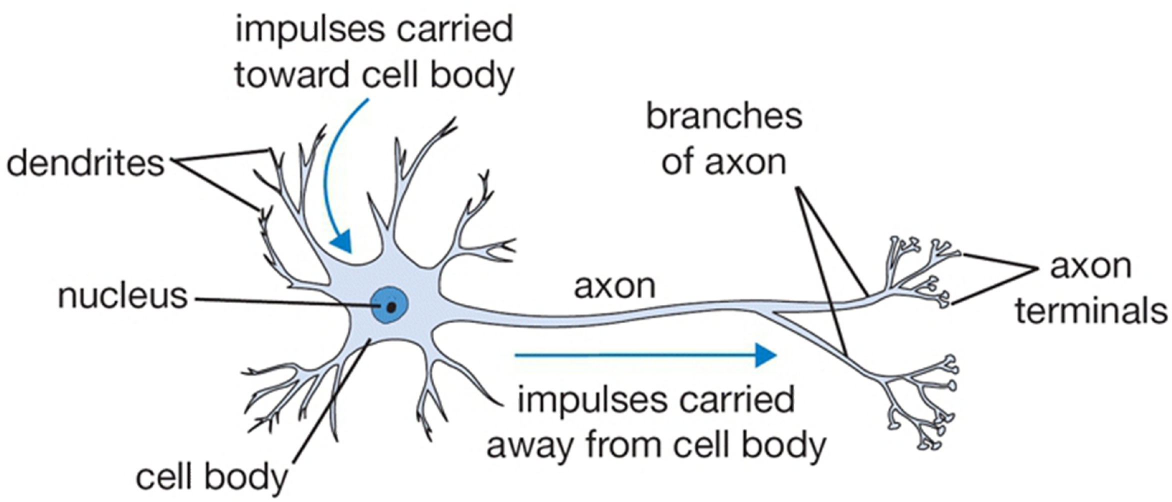


Figure1.6: Neuron (Kandel, et al. 2000)

Cell Body or Soma:

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

Axon:

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

Dendrites:

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

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. **(**Howland and Mycek,et al. 2006).

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,et al. 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).

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, (Longstreth et al.2006).

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) (Longstreth et al.2006).

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"

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 (Timbrell et al.2006).

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, et al .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, et al. 1980).

1.4.4. Hematology Introduction:

An analysis of blood was exercised from far back to ancient times. All three blood cell types performs its own role in healthy men's life and so count of different cell type of blood can

identify different diseases that's the reason that complete blood cell count is the most common test carried out in all clinical laboratories. Different techniques were practiced since the discovery of blood cells in 1658. Before going into details of modern blood cell counting methods we should know the history of cell counting and the developments in the technology of cell counting which was finally implemented to quantification of the ingredients of blood.

1.4.4.1. Hematology

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

1.4.4.2 History of Cell counting

Leeuwenhoek was the first person who attempted to count blood cells using a glass capillary tube with graduation marks of measured dimension and microscope to count. He selected chicken to count red blood cells 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 cells. [Hajdu, SI et al .1998;42:1075].

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, et al. 2013).

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; CO_2 in large amounts (about 97%) and O_2 in small amounts (about 3%), various nutrients (glucose, fats), wastes of metabolic exchange (urea, ammonia), hormones, and vitamins.

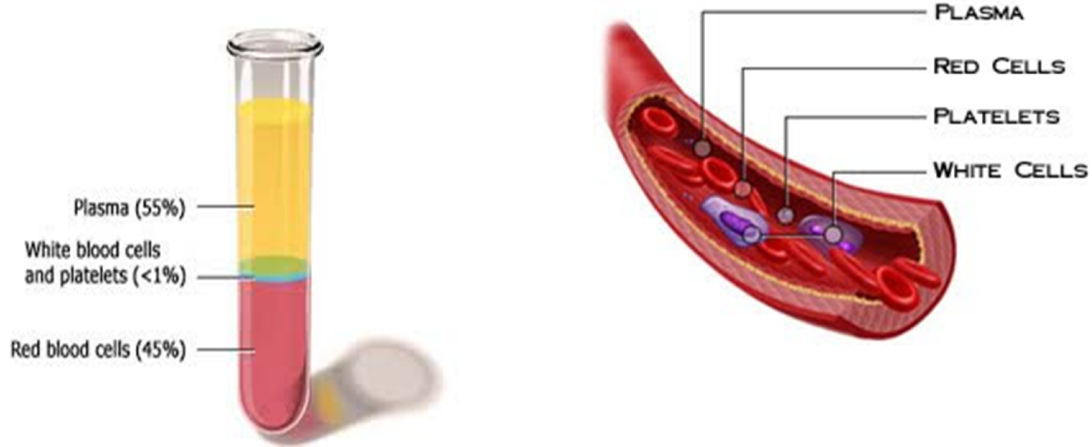


Figure1.7: Plasma of the Blood (Alberts, et al. 2013)

Red Blood cell *(Erythrocytes)*:

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

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

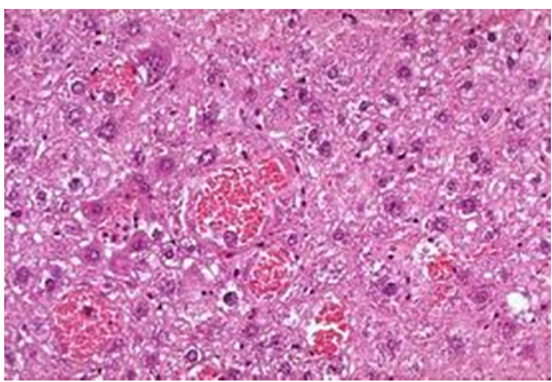


Figure 1.8: Red Blood cell (Alberts et al. 2013)

- **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 vertebratesas 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. (Alberts, et al. 2013)

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(Alberts, et al. 2013))

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. (Alberts, et al. 2013)

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. (Alberts, et al. 2013)

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 et al. 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. (Alberts, et al 2013)

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). (Alberts, et al 2013)

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, et al 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 inhypochromic 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. (Alberts, et al 2013)

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. (Alberts, et al 2013)

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.

Red blood cell distribution width (RDW or RCDW)

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

Higher than Normal RDW

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

1.4.7. White Blood Cell

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

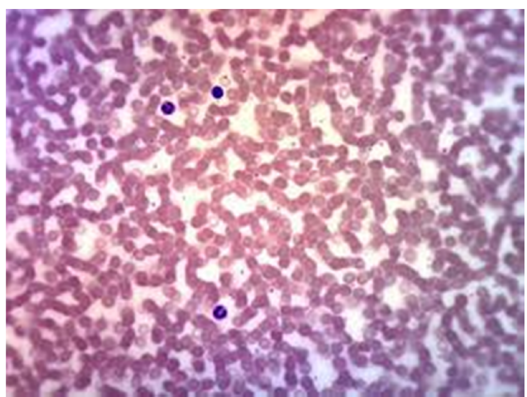


Figure 1.9: White Blood Cells (Alberts, et al 2013)

- **Normal range of WBC: 3-7×10^3mm^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. (Alberts, et al 2013)

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, et al 2003)

- **Normal range of platelet: 1000-1600×10^3mm^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 ofintestinal 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 (Burcham. PC et al, 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 emulsificationof 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. (Ganong, et al 2003)

Figure 2:Liver (Ganong, et al 2003)

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. (Ganong, 2003)

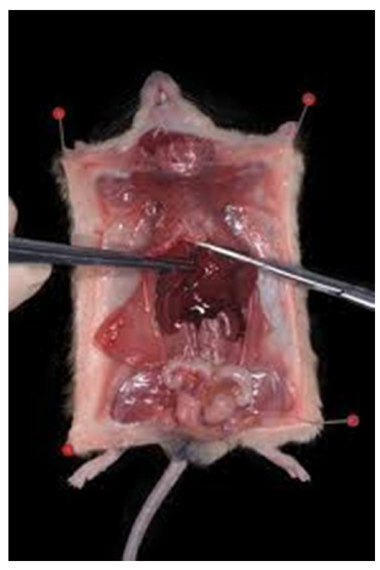


Figure3.1 Anatomy of mice (Ganong, et al 2003)

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. (Ganong, 2003).

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) (Ganong, 2003)

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. (Ganong, 2003)

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. (Ganong, 2003)

- **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) (Ganong, 2003)

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. **(Ganong, 2003)**

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 highe (Ganong, 2003).

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. (Ganong, 2003).

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 (Ganong, 2003).

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. 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 (Bennett et al., 2000).

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 (Khan et 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 antiaggregatory 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

(Gaon et al., 2003).

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 (Lobo V et al., 1991).

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
Plant
Introduction

2 . Plant Introduction

2.1 vernacular name:

Table 1: showing the vernacular names of *Grewia asiatica* Schott in different regions

Other vernacular names
BENGALI: Phalsa, Shunkri.
FRENCH: Raisin de l'Inde, Raisin des Philippines, Raisin falsa.
GUJARATI: Shukri.
HINDI: Phalsa, Phalsa chhaal.
KANNADA: Dagala, Dadasala, Phulsha.
KHMER: Pophliё.
LAOTIAN: Nhap.
MALAYALAM: Chadicha.
MARATHI: Palshi, Phalsa.
ORIYA: Pharosakoli.
PUNJABI: Faalsaa.
RUSSIAN: Falsa.
SANSKRIT: Alpasthi, Mriduphal , Parapara, Paroushak, Parushaka.
SPANISH: Falsa.
TAMIL: Palicamaram , Unnu.
TELUGU: Phutiki.
THAI: Dton mai laai, Lai khon, Ma laai, Po tao hai, Yap khee thao.
URDU: Phalsa.
VIETNAMESE: Cò ke á, Giam phủ, Cò ke pierre.

2.2Taxonomy:

Scientific classification

Kingdom: Plantae

(Unranked): Angiosperms

(Unranked): Eudicots

(Unranked): Rosids

Order: Malvales

Family: Malvaceae

Subfamily: Grewioideae

Genus: *Grewia*

Species: *G. asiatica*

Binomial name

Grewia asiatica L.

Synonyms

Grewia hainesiana Hole

Grewia obtecta Wall. [Invalid]

Grewia subinaequalis DC. [1]

Figure: 2.1 whole plant

Figure: 2.2 fruit

Figure: 2.3 stem

2.3Plant information

The dieto-therapeutic importance of fruits is well-recognized since the beginning of human civilization. Fruits occupy an important position in the socio-cultural and health systems of many countries and there is a growing interest in exploring their therapeutic and nutritional properties.

Antioxidants present in fruits and juices made from them, are claimed to be helpful against cancer, cardiovascular and various chronic diseases. The presence of various biofunctional and chemo-preventiv compounds in fruits, believed to have health-boosting properties, are a major reason for their increasedconsumption. Fruits are regarded as a valuable food commodity with potential health benefits, being arich source of carbohydrates, vitamins, antioxidants and minerals which are essential for an active and healthy life.

Grewia genus (*Tiliaceae*) comprises approximately 150 species of small trees and shrubs, distributed in subtropical and tropical regions of World and is the only ge*G. asiatica*L., *G. damine* Gaertn., *G. elastica* Royle, *G. glabra* Blume, *G. helicterifolia* Wall., *Grewia microcos* L.,*G. optiva* J. R. Drumm. ex Burret, *G. sapida* Roxb., *G. tenax* (Forssk.) Fiori and *G. villosa* Willd..*Grewia asiatica* L., grows wildly, and is also cultivated in south Asian countries. The name *Grewia*was given due to Nehemiah Grew, one of the founders of plant physiology, while *asiatica* reflects theAsian origin of this species.*G. asiatica*, locally known as *phalsa*, is well-known for its nutritional andtherapeutic attributes. Despite its diverse use, it has suffered notable disregard, as is evident from thelack of literature on this plant. As a step in this direction and as part of our studies on documentingbiological and chemical studies of indigenous flora of Pakistan, we have reviewed the phytochemistry nutritional importance and pharmacological properties of this plant. This review will serve as a usefulreference for further Botanical Description and Traditional Uses

Grewia asiatica is a 4 to 5 m tall shrub. The leaves are approximately 5–18 cm long and broad. Theflowers are arranged in cymes of several together, the individual flowers are yellowish in color withfive large (12 mm) sepals and five smaller (4–5 mm) petals. The flower has a diameter of about 2 cm.*Grewia asiatica* L., FL, flower; L, leaf, S, seed.Leaves may be ovate,suborbicular, acute, subacuminate or cuspidate, sharply and often coarsely,double serrate, subglabrous above, hoary-tomentose beneath and rounded or only slightly cordate atthe base. Flower buds are broadly cylindric or clavate, peduncles are axillary, usually many, and longand slender. Bracts are present beneath the pedicles. The fruit is globose, 1.0 to 1.9 cm in diameter, 0.8to 1.6 cm in vertical height, and 0.5 to 2.2 g in weight and is edible portion of plant. Plant flowersappear in January-February and fruits mature in May-June. During ripening, the fruit skin turns fromlight green to cherry red or purplish red, becoming dark purple or nearly black when fully ripened. The ripe fruit is soft and delicate and is covered with a very thin whitish blush. The fruit is like aberry and has a sweet and sour acidic taste. The flavor is like grapes. Phalsa fruits fetch a very good price of $1/Kg in local markets and are consumed fresh with some salt, which is added to increase palatability. It is also used in making jams, pies, squashes and chutneys. However, it has a short shelf life and is considered suitable only for local marketing.The fruits are claimed to be beneficial for heart, blood and liver disorders, anorexia, indigestion, thirst, toxemia, stomatitis, hiccough, asthma,spermatorrhoea, fevers and diarrhea and are used for treating throat, tuberculosis and sexual debility troubles. The root bark is used for the treatment of

rheumatism and urinary tract problems , while the stem bark is used in sugar refining . The leaves are applied to the skin for wounds and cuts and to relieve irritation and painful rashes.

Two distinct types, tall and dwarf, have been developed in India that differ with respect to various chemical and physical characteristics (Table2. 1). The juice yield is slightly higher in the tall type because it is directly related to edible portion, while more total sugars and non-reducing sugars were observed in the dwarf type. Tall type had more reducing sugars and titrable acidity and a greater amount of seed protein than the dwarf type. (Ganong, 2003)

Table2 1.Characteristics of tall and dwarf types of *Grewia asiatica*

Content (%)	Tall	Dwarf
Edible portion	91.30	90.79
Seed	8.70	9.21
Juice yield	67.50	65.90
Pomace	32.50	34.10
Moisture	76.80	74.83
Total sugars	5.73	7.95
Reducing sugars	1.24	0.99
Non-reducing sugars	4.49	6.96
Titrable acidity	1.48	1.12
Fruit protein 3.13 1.89	3.13	1.89
Seed protein	8.75	7.00
Pulp protein	1.40	7.00

2.3. Compositional and Phytochemical Studies

2.3.1. Compositional Studies

Fruits of *G. asiatica* are low in calories and fat, and high in vitamins, minerals, and fiber. The detailed nutritional profile of fruit has been given in Table 2.

Table 2.2.Nutritional values of fruit

Nutrients Values/100 g g
Protein (g) 1.57
Total lipid (fat) (g) <0.1
Carbohydrate (g) 21.1
Ash (g) 1.1
Fibre (g) 5.53
Calcium (mg) 136
Iron (mg) 1.08
Phosphorus (mg) 24.2
Potassium (mg) 372
Sodium (mg) 17.3
Vitamin B1 (mg) 0.02
Vitamin B3 (mg) 0.825
Vitamin C (mg) 4.385
Vitamin B2 (mg) 0.264
Vitamin A (g) 16.11

All these components are essential for healthy life. Vitamin C helps in wound healing and collagen formation and increases iron absorption from meal. Presence of sufficient amount of fibre helps to lower chances of obesity, cardiovascular disease, diabetes and certain cancers. In a recent study,various standardization parameters of fruits, microscopical and macroscopical characters, andphysicochemical parameters like total ash (5.16%), water soluble ash (2.5%), acid insoluble ash (1%),sulphated ash (0.66%), loss on drying (8.3%), swelling index (0.1) and foaming index (less than 100) have been evaluated. Successive extraction and cold maceration values of fruits (%; w/w) in various solvents like petroleum ether (1.6 and 3), benzene (2.1 and 3.3), chloroform (2.6 and 4), ethyl acetate 3.7 and 4.3) and methanol (30.9 and 45.6) were observed, respectively. Phytochemical screening revealed the presence of alkaloids , glycosides, proteins and amino acids, saponins,steroids, acids, mucilage, fixed oils and fats. Fruits were observed for their characters under visible andultralviolet light after treating with different chemical reagents and pharmacognostic parameters variable on the basis of their geographical origin were determined. Previous pharmacognostic studies of fruit indicated extractive values for petroleum ether, benzene, ethyl acetate, methanol and distilled water as 0.6, 1.3, 1.5, 34.5 and 12.5%, respectively.

Total ash, acid insoluble ash and water soluble ash were 3.0, 1.4 and 1.1%, respectively. The behavior of the powdered leaves with different chemical reagents was noted. Fluorescence characteristics of the powdered leaves and extract wereunder UV (254 and 366 nm) and visible light.

The fruits are excellent for making juice and squash, which are regarded as very nutritious beverages by indigenous people. A refreshing summer drink prepared from fruits, called *phalsay kasharbat* is available in food-stores and is believed as a cardiac tonic. The fruit juices may be fortifiedwith other nutrients to further enhance its nutritional contribution to the diet. The juice has a lowindex and may be taken to manage diabetes, because carbohydrates in low glycemic indexfoods break down more slowly. Moreover, low glycemic index foods are believed to lessen the risk ofcoronary heart disease, and obesity. Nutritionally essential amino acids such as threonine andmethionine are present in pulp and seeds, respectively, whereas phosphoserine, serine, and taurine arethe dominant amino acids in juice. The pulp contains higher concentrations of phosphoserine ascompared to other free amino acids, while the hydrolysed product contained aspartic acid, glycine, andtyrosine in large amount. Pigments and total soluble solids have been obtained from pomace. Thehighest yield of pigments and total soluble solids were obtained by addition of water (75%) topomace . A ready-to-serve (RTS) beverage from the fruit juice was formulated and standardized. Itcontained 25% juice and a Brix-acid ratio of 45:1 .A syrup was prepared from fruit juice, mixingthe clear juice with an equal amount of sugar and preserved with sodium benzoate. Amino acidswere measured in the hydrolyzed and unhydrolyzed (free) pulp and seed and related to determinationof the degree of adulteration in fruit juice. Threonine was found in pulp but was missing in seedextract, whereas methionine was only present in seeds, indicating that the presence of methionine infruit juice would be the result of adulteration. Phosphoserine, serine, and taurine were the dominantamino acids in juice . *G. asiatica* fruits were analyzed for six micronutrients (Co, Cr, Cu, Ni, Zn
and Fe) on fresh weight (FW) and dry weight (DW) (Table 3) . Iron was present in the highest concentration, while cobalt was present in the lowest amounts. Micronutrients play an important role in various physiological and metabolic processes of the human body.

Table 3.Mineral contents of fruit .

Mineral	mg/100 g FW	µg/100 g DW **100 g DW **
Cobalt	0.99	33
Chromium	1.08	36
Copper	0.48	16
Nickel	2.61	87
Zinc	144	48
Iron	140.8	1695

* FW = Fresh weight (Fresh fruit); ** DW = Dry weight (After removal of moisture from fresh fruit).

Chemical composition of seeds indicated that they contain bright yellow oil (5%). Fatty acid composition of this oil indicated the presence of palmitic (8%), stearic (11%), oleic (13.5%) and linoleic acids (64.5%) while small amount of unsaponifiable matter (3%) was also detected. .(Parveen, et al,.2012)

2.3.2. Phytochemistry

. Preliminary Phytochemical Screening and Primary Metabolites Phytochemical screening of the leaves revealed that their petroleum ether extract contains diterpenes, glycosides and fats; chloroform extract contains alkaloids and glycosides, while ethanolic extract contains triterpenoids, sterols, flavonoids, saponins and tannins. Pharmacognostic evaluation of leaves reported total 5% of ash, consisting of water-soluble ash (2.5%) and acid-insoluble ash (2.1%). Other studies reported the extractive values of leaves in various solvents: petroleum ether (1.2%), benzene (1.2%), chloroform (1.6%), ethyl acetate (1.8%) and methanol (13.6%) .Phytochemical screening of fruits indicated the presence of carbohydrate, tannins, phenolic compounds,flavonoids and vitamin-C in methanolic extract; flavonoids and fixed oil in petroleum ether extract; steroids in benzene extract; carbohydrate, tannins, flavonoids and phenolic compounds in ethyl acetateextract and carbohydrate, tannins, phenolic compounds and proteins in the aqueous extract Amino acids such as proline, glutaric acid, lysine and phenylalanine, and carbohydrates, like glucose,xylose, and arabinose were identified by paper chromatography in ethanol extract of fruit.

2.4.Compounds Isolated and Secondary Metabolites

Fruits of *G. asiatica* contain pelargonidin 3,5-diglucoside, naringenin-7-*O*-β-D-glucoside, quercetin,quercetin 3-*O*-β-D-glucoside, tannins, catechins, and cyanidin-3-glucoside [40]. Grewinol and itsderivatives were isolated from the dried flowers .Similarly β-sitosterol, quercetin, quercetin 3-*O*-β-D-glucoside, naringenin, naringenin 7-*O*-β-D-glucoside and a δ-lactone 3,21,24-trimethyl-5,7-dihydroxyhentriacontanoicacid were isolated from flowers. The stem bark contains betulin, lupeol,lupenone, and friedelin. β-amyrin and β-sitosterol were isolated from heartwood of *G. asiatica* Quercetin, kaempferol and a mixture of their glycosides were isolated from leaf extracts Citricacid trimethyl ester, α-methyl-l-sorboside , stigmasterol, campesterol and 9,12-octadecadienoic acidmethyl ester were the main compounds identified in *G. asiatica* pomace extractCompounds isolated from *G. asiatica*:

Table 4.Compounds isolated from *G. asiatica*:

Serial no	Compounds isolated
1.	Pelargonidin 3,5-diglucoside (1),
2.	Naringenin-7-*O*-β-D-glucoside(2),
3.	Quercetin (3),
4.	Quercetin 3-*O*-β-D-glucoside (4),
5.	Catechins (5),
6.	Cyanidin-3-glucoside (6),
7.	Grewinol (7),
8.	Naringenin (8),
9.	3,21,24-trimethyl-5,7-dihydroxy-hentriacontanoic acid δ lactone (9),
10.	Betulin (10),
11.	Lupeol (11),
12.	lupenone (12),
13.	Friedelin (13),
14.	α-Amyrin (14),
15.	β-amyrin (15),
16.	β-sitosterol (16),
17.	Lanost-9(11)-en-12-one (17),
18.	Docosanol (18),
19.	Nonacosanol (19),
20.	Stigmast-7-en-3-ol (20),
21.	Citric acid trimethyl ester (21),

22.	α-methyl-l-sorboside (22),
23.	stigmasterol (23)
24.	Campesterol (24),
25.	9,12-octadecadienoic acid methyl ester (25)

.(Parveen, et al,.2012)

2.5 Plant descripton

Falsa or Phalsa is a small berry which grows in a small tree and native to India and South Asia. This fruit with Latin name *Grewia aslatica* now is being widely cultivated in tropical countries. Most of the falsa's farmers grow its trees where there is the land which has loamy soils. Also, the taste of falsa fruit is incredible which has a combination of sour, bitter, and acidic taste. Then, the texture is similar to grapes due to the presence of a white color pulp inside. Further, falsa fruit belongs to berry family.

It is known that this fruit has many great purposes to help human's life. The wood is being used as golf sticks, poles, and singles caused by the strong and elastic form. Not only providing that function but also the bark has a role as a good ropes material to make. Then, falsa fruit is such a natural and beneficial fruit to help with many kinds of purposes.

As the consequence, consuming good foods is a must. One thing that is also important is how we pick the best food to fulfill the nutrient needs perfectly. Then, there are many options to choose. Moreover, in this article, we will describe a fruit which is excellent in nutrients contained. Yes, it is falsa fruit which gives you a great option to eat.

2.6Nutrients Value of Falsa Fruit

Then, before going to health benefits of falsa fruit section, it is best to have a look the nutritional value of falsa fruit below.

Information	Amount per 100g
Calories	90.5 Kcal
Protein	1.57 g
Carbohydrates	21.1 g
Calcium	136 mg

Dietary Fiber	5.53 g
Phosphorus	24.2 mg
Iron	1.08 mg
Potassium	372 mg
Sodium	17.3 mg
Vitamin A	16.11 µg
Vitamin B1	0.02 mg
Vitamin B2	0.264 g
Vitamin B3	0.825 g
Vitamin C	4.38 g

*Percent Daily Values are based on 2,000 calorie diet. Your Daily Values may be higher or lower depending on your calorie needs. .(Parveen, et al,.2012)

1. **Source of Antioxidants**

As a fruit which has a great source of antioxidants, falsa fruit is having a role in maintaining the body health. Moreover, if you want to eat other fruits which are rich in antioxidants, try to have orange, strawberries, lettuce as options.

2**. Source of Sodium**

As you can see on the table above, falsa fruit has a great source of sodium. Then, what is the function of sodium in our body? Well, it acts as an electrolyte and essential ion to promote the enzymes operation and muscle as well. Moreover, it also increases the performances of a nervous system and is needed for blood regulation. Hence, it is very best option to have falsa fruit as your number one sodium's food source.

3. Source of Vitamin A

With the high concentration of vitamins contained in falsa fruit, it will boost your health for sure. Like other nutrients do, Vitamin A is a great natural nutrient that helps to promote good vision and prevents macular degeneration.

4. Source of Vitamin B1

Vitamin B1 which presents in falsa fruit helps to maintain the heart and nerve function. In contrast, the deficiency of vitamin B1 consumption may lead to certain health problems including anorexia, muscle weakness, and nerve damage.

5. Source of Vitamin B2

Vitamin B2 as known as Riboflavin is having a role in promoting blood cells and the body metabolism. To fulfill the needs of vitamin B2, you can consume almonds, egg, and spinach as well.

2.7Cultivation:

Phalsa grows both in tropical and subtropical climates. However, is does best in regions having distinct summer and winter seasons, withstanding short periods of light frost as well as temperatures as high as 44* C. In the absence of a prominent change of seasons the plant does not shed its leaves, flowers erratically throughout the year and fruits poorly. This limits its distribution in South-East Asia. Phalsa can be grown on a wide range of soils, even those which are moderately alkaline.

Seeds germinate in 15-20 days. The juvenile phase lasts for 15-18 months. Flowers develop only on new shoots of the current growing season. The period between flowering and fruit maturity is 45-55 day. Under subtropical condition there is a brief period of dormancy lasting 4-6 weeks when the plant sheds its leaves. There are no distinct cultivars, but the cultivated plants are usually of a dwarf shrubby type while the wild plants are tall. Sometimes nurserymen give fancy names to plants they claim to be superior, in an effort to enhance their Propagation is mostly by seed which does not require any pre-sowing treatment. Seedlings are ready for planting out within 3-4 moths. Asexual propagation by cutting, layers and budding is feasible. Planting is usually done during the monsoon months though dormant planting is also possible. The planting distance is 2.5 m x 3 m Phalsa is deciduous and requires annual pruning to stimulate the emergence of numerous shoots of moderate vigour

which flower and fruit. Leafless plants are cut back to a height of 60-90 cm after heavy Manuring with farmyard manure. The new shoots should not suffer water shortage until he harvest in May (April-June in India); irrigation may be essential during this period. There is no serious pest or disease of this species.The small fruits have to be hand-picked every morning throughout the harvesting season, which lasts for about three weeks. This is very time –consuming operating and can create difficulties where labour is scarce. Pre-harvest ethephon sprays (concentration 100 mg/1) are very effective in inducing simultaneous ripening but the treated fruits lack natural taste and flavour. The annual yield is 3-5 kg per plant or 4.5-6 t/ha. .(Parveen, et al,.2012)

2.8 Medicinal use:

he fruits have many number of medical applications, the paste of the leaves cures skin ailments like eczema, eruptions, heals and wounds,

the fruits rawer part helps in curing blood disorders, fevers, inflammations, purification of blood also acts as a medicine for respiratory problems such as asthma, bronchitis, colds, coughs and sore throat

It solves urinary problems such as burning and their juice helps ease the problem of high acidity and indigestion.

The fruit controls the blood pressure and cholesterol also soothing liver and gall bladder problems,

The seeds are used for treating gonorrhea and fertility related problems, also safeguards from sunstroke and heals anemia .

The barks infusion is used in the treatment of rheumatism and diarrhea .(Parveen, et al,.2012)

Chapter -3

Literature review

LITERATURE REVIEW

1 Radioprotective Potential of *Grewia asiatica* Fruit Extract in Mice Testis: GAE have the protective potential to ameliorate the damaging effect of radiation to the testis. (Sharma et al,2007)

2 Preliminary Phytochemical and Hypoglycemic Activity of Leaves of *Grewia asiatica* L.:

Ethanol extracts (200mg/kg b.w. p.o.) shown more significant ($p<0.01$) reduction in blood glucose level in alloxan induced diabetic Wister rats compared to control and glibenclamide as standard (10 mg/kg b.w. p.o.). And all extracts also screen for phytochemical study revealed presence of Triterpenoids, Alkaloids, Flavonoids, Sterols, Tannins (priyanka et al, 2000)

3 Antihyperglycemic Potential of *Grewia asiatica* **Fruit Extract against Streptozotocin-Induced Hyperglycemia inRats:**

Oral administration of fruit, stem bark and leaves reduced serum glucose level of alloxan induced diabetic rabbits. The results suggest that the fruit, stem bark and leaves of Grewia asiatica have shown significant antihyperglycem. (Khattab et al., 2007)

4.Antihyperglycemic Potential of *Grewia asiatica* **Fruit Extract against Streptozotocin-Induced Hyperglycemia inRats: Anti-Inflammatory and Antioxidant Mechanisms:**

The treatment also protects against STZ-induced pathological changes in the pancreas. Theresults of this study indicated that *G. asiatica* fruit extract exerts antihyperglycemic activity against STZ-induced hyperglycemia.The improvement in the pancreatic β-cells and antioxidant and anti-inflammatory effects of *G. asiatica* fruit extract may explain the antihyperglycemic effect , (Khattab et al., 2007)

5. Hepatoprotective efficacy of *Grewia asiatica* fruitagainst oxidative stress in swiss albino mice:

Husbiochemical and histopathological results proves that GAE has the potential against radiation (.sharma et al, 2007)

6. Evaluation of immunomodulatory activity of *Grewia asiatica* inlaboratory animals:

The present study clearly indicated immunostimulant property of GAEE and lends support for further development of new immunostimulant drug.(Sharma et al,2004)

7. Evaluation of the free radical scavenging activity and radioprotective efficacy of *Grewia asiatica* fruit:

noticed. GAE was found to have strong radical scavenging activity in 2,2-diphenyl-1-picrylhydrazyl (DPPH∗) and O−2 assays and also showed *in vitro* radioprotective activity in proteincarbonyl assay in a dose-dependent manner. The above results prove theradioprotective efficacy of GAE.(shukla et al,2016)

Chapter-4
Materials and Methods

4.1.1Collection of plant

The plant was collected from Foridpur district of Bangladesh. A voucher specimen (Accession number: 38479) 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.

Figure-:4.1 Herbarium sheet of *Grewia asiatica*

4.1.2Drying

Drying is the most common and fundamental method for post- harvest preservation of medicinal plants because it allows for the quick conservation of the medicinal qualities of the plants material in an uncomplicated manner. The plant was dried in room temperature and drying was completed within 2 to 3 weeks. By drying the plant materials one removes sufficient moisture to ensure good keeping qualities and to prevent molding the action of enzyme, the action of bacteria and chemical or other possible changes. Proper and successfuldrying involved two main principles: control of temperature and regulation of air flow. The plant material can be dried by room temperature.

4.1.3 Cutting, Grinding and Sieving

The stem was cut into small pieces and placed in the grinding machine to make it fine powder. As the stem is too hard, very small amount of pieces were placed in the hopper with an average rotation. The grinded powder was collected very carefully.

4.1.4Blending

Finally the powder was placed in blender machine in order to get more fine powder. During the entire blending process precautions were taken to avoid all kind of cross contamination and product loss.

4.1.5 Preparation of plant extraction

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

At first 3kg dried plant dust of *Grewia asiatica* was soaked in L methanol in12 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 53 degree Celsius temperature. The number of rotation per minute was selected as 125 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.

4.1.6 Crystal formation

After completing rotary crystal formation was occurred that was good in amount. These crystals are clear and stable. These crystals are not soluble in polar and not polar solvent and intermediate solvent. Further investigation will be continued to know about these crystals.

Figure-4.2: Formation of crystals from crude extract

Figure 4.3 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 4.4.*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. CNS Activity Test

4.5.1. Materials for CNS Activity Test:

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

4.5.2. Chemical Agents Used in CNS activity Test:

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

4.5.3. 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.

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

4.5.4.1. Open Field Test:

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

4.5.5. Methods for CNS Activity Test:

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

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

4.5.5.1. 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 ***Grewia Asiatica*** 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 4.5: Open Field Test

4.6. Toxicity Test

4.6.1 Materials for Toxicity Test

- Analytical Balance,
- Feeding needle: 1 c.c.
- Insulin syringes 100 units disposable
- 5 ml syringe disposable
- Dissecting box
- Dissecting pad
- Pin
- Beaker 1 litre
- Petri dish for washing
- Epindrop tube
- 250 ml food grade plastic pot
- Gloves
- Mask

4.6.2 Chemical Agents Used Toxicity Test

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

4.6.3 Doses Used for Toxicological Activity of the Extract:

4.6.3.1. Acute Toxicity Test:

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

4.6.3.2. Chronic Toxicity Test:

Methanolic extracts of***Grewia Asiatica*** 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.

4.6.4 Methods for Toxicity Test:

4.6.4.1. 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 behaviour.(Paul, et.al. 2012).

4.6.4.2. Chronic Toxicity Test

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

4.6.5. Hematological parameters

Collected blood was used for the estimation of hemoglobin (Hb) content; red blood cell count (RBC) and white blood cell count (WBC).(Estimations are carried out by using the **Sysmex**

XT 2000i Hematology Analyzer ,CARe Specialized Hospital and Research Centre Ltd, Dhaka, Bangladesh)

4.6.6. Serum biochemical parameters

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

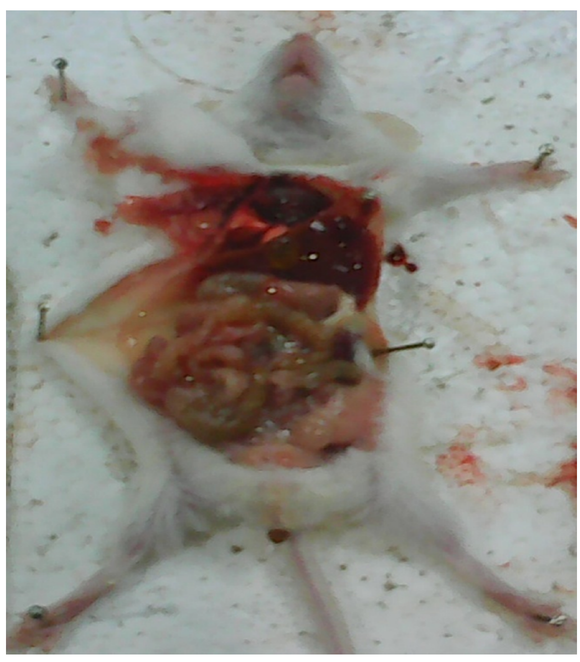


Figure 4.6. Mice Organ

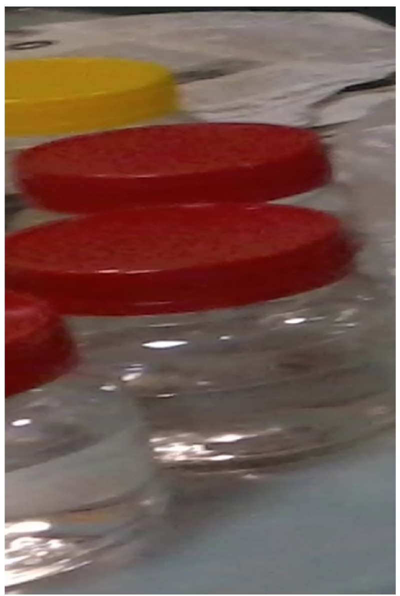
4.6.7. Histopathological studies

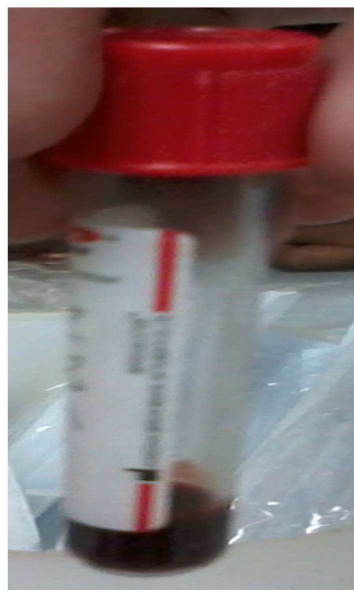
After sacrifice the organs like heart, lung, liver, kidney and pancreas of animals from each group were subjected for histopathological examinations. After fixing the tissues in 10% formaldehyde the tissues were dehydrated and paraffin blocks were made. Then sectioning was done at about 5-7μ. Routine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

4.6.8. 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.





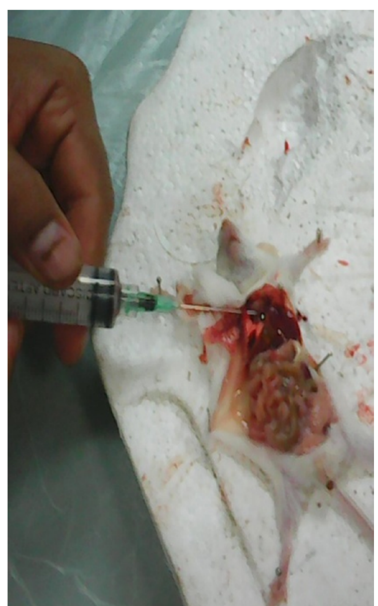


Figure 4.7. Collecting the blood and organ of mice

4.7. Antioxidant

4.7.1 Total phenolic Content Assay

4.7.1.1. 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.

4.7.1.2. Principle

In the alkaline condition phenols ionize completely. When Folin-Ciocalteu reagent isused in this ionized phenolic solution the reagent will readily oxidize the phenols. Usualcolor of Folin-Ciocalteu reagent is yellow and after the oxidation process the solutionbecome 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.

4.7.1.3. 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.

Table4.1: 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

4.7.1.4. 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.

4.7.1.5.Sample Preparation

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

4.7.1.6. 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

4.7.2.. DPPH Radical-Scavenging Activities

4.7.2.1. 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.

4.7.2.2. 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.

4.7.2.3. 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.

4.7.2.4. 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.

4.7.2.5. 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.

4.7.2.6. 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.

4.7.2.7.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.

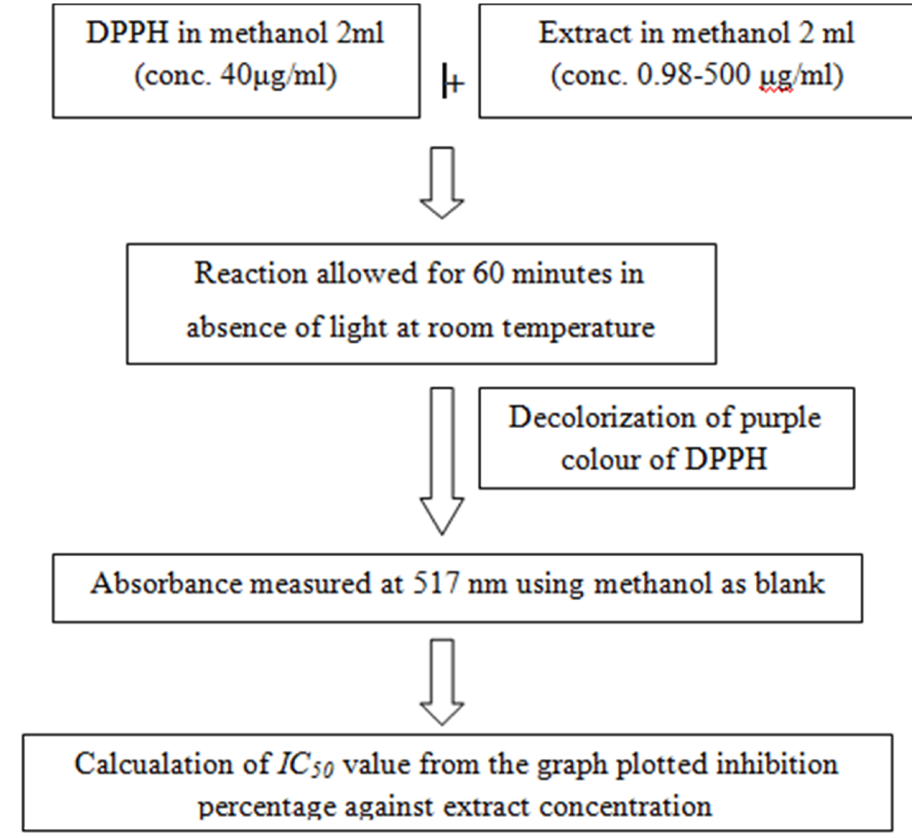


Figure 4.8.: Schematic representation of the method of assaying free radical scavenging activity.

4.7.3. Reducing Power Assay

4.7.3.1. Introduction

Free radicals are types of Reactive Oxygen Species (ROS), which include all highly re active, oxygen□
containing molecules. Types of ROS include the hydroxyl radical, the super oxide anion radic al, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and various l

ipid eroxides. These free radicals may either be produced by physiological or biochemical pro cesses or by pollution and other endogenous sources. All these free radicals are capable of rea cting with membrane lipids, nucleic acids, proteins and enzymes and other small molecules, r esulting in cellular damage

Antioxidants prevent the human system by neutralizing the free radicals interactively and syn ergistically. Plants arerich source of free radical scavenging molecules such as vitamins, terpe noids, phenolic acids, lignins, stilbenes, tannins, flavanoids, quinones, coumarins, alkaloids, a mines, betalains and other metabolites which are rich in antioxidant activity.

4.7.3.2. 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.

4.7.3.3. Material and Method

Reducing power assay of crudeextracts 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.

4.7.3.4. 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/µlten 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/mlto 500 µg/ml. The test was done triplicate.

4.7.3.5. 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/µlten 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.

4.7.3.6. 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.

4.5. 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 *H.Kurzii* 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.

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 5

Result and Discussion

5. Result and Discussion

5.1.*Grewia asiatica*:

Crude extract of *Grewia asiatica* 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*Grewia asiatica*(leaf). Table-5.1 and figure-5.1 show the laxative effect by charcoal meal GI transit test of the crude extract of *Grewia asiatica* 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. 400 mg/kg, & 800mg/kg, and 800mg/kg + atropine of crude extract *Grewia asiatica* were used for the gastrointestinal transit test.

Table 5.1: Effects of crude extract of) *Grewia asiatica* 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	54.17±2.96
5.	Dose-2 male, (p.o)	800	70.43±2.93
6.	Dose-2 female(p.o)	800	70.62±1.89
7.	CMCmale (p.o)+Atropine (i.p)	10+10	46.5±5.1
8.	CMCfemale (p.o)+Atropine (i.p)	10+10	46±0.00
9.	Dose-2 male(p.o)+ +Atropine (i.p)	800+10	56.66±4.1
10.	Dose-2 female(p.o)+ +Atropine (i.p)	800+10	54.54±0.01

Dose-1=400mg/kg (*Grewia asiatica)*

Dose-2= 800 mg/kg (*Grewia asiatica)*

All the test group of crude extract except group-3 showed significant increased transit ($p<0.001$, $p<0.01$ and $p<0.05$) of GI motility test at a dose of 400 mg/kg, 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 (group-6) showed highest percentage of the

intestinal length traversed by the charcoal (**70.62±1.89,** 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. Crude extract of group 7 showed most prominent percentage of the intestinal length traversed by the charcoal, that is 56.66±4.10 (**,** p<0.001) and the reference drug showed the percentage of length (74.67±1.20**,** p<0.001).

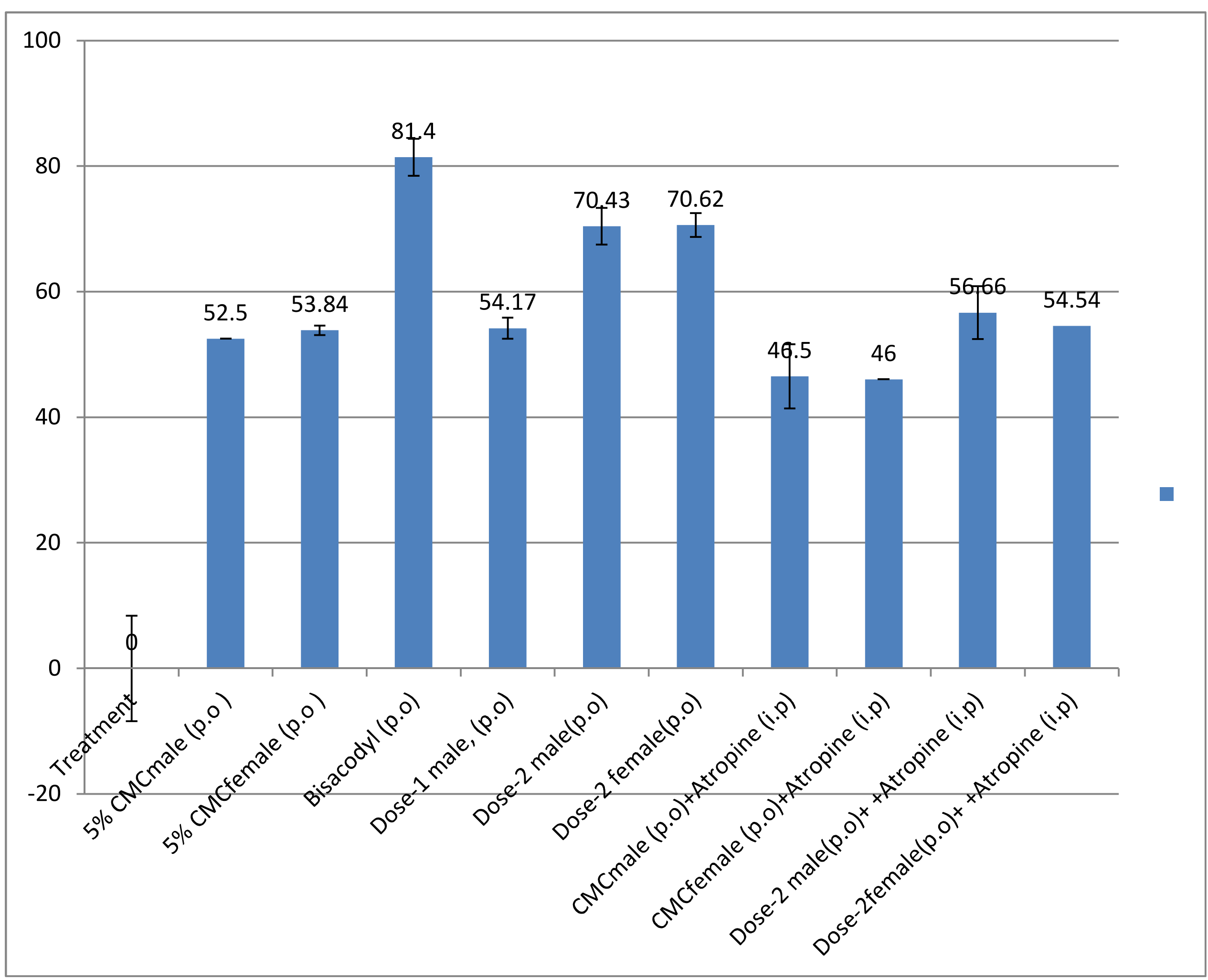


Figure 5.2: Bar diagram showing the dose-dependent effect of crude extract on the travel length (charcoal meal through small intestine of mice, in the absence and presence of atropine).

5.2.CNS Activity Test of Methanolic Extract of *Grewia asiatic*

Table 5.2: CNS Activity of plant extract of *Grewia asiatica*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 Grewia asiatica	200mg/kg	61.900±9.908	36.100±5.920	24.600±5.081	23.600±4.987	23.600
Crude extract of Grewia asiatica	400mg/kg	59.00±8.951	26.200±5.12	23.700±5.450	22.200±3.797	39.500
Crude extract of Grewia asiatica	800mg/kg	93.400±7.817	30.700±6.711	22.700±3.428	18.200±2.905	52.300
Positive control, Diazepam	1mg/kg	121.83±1.1	69.33	53	35.67	27.83

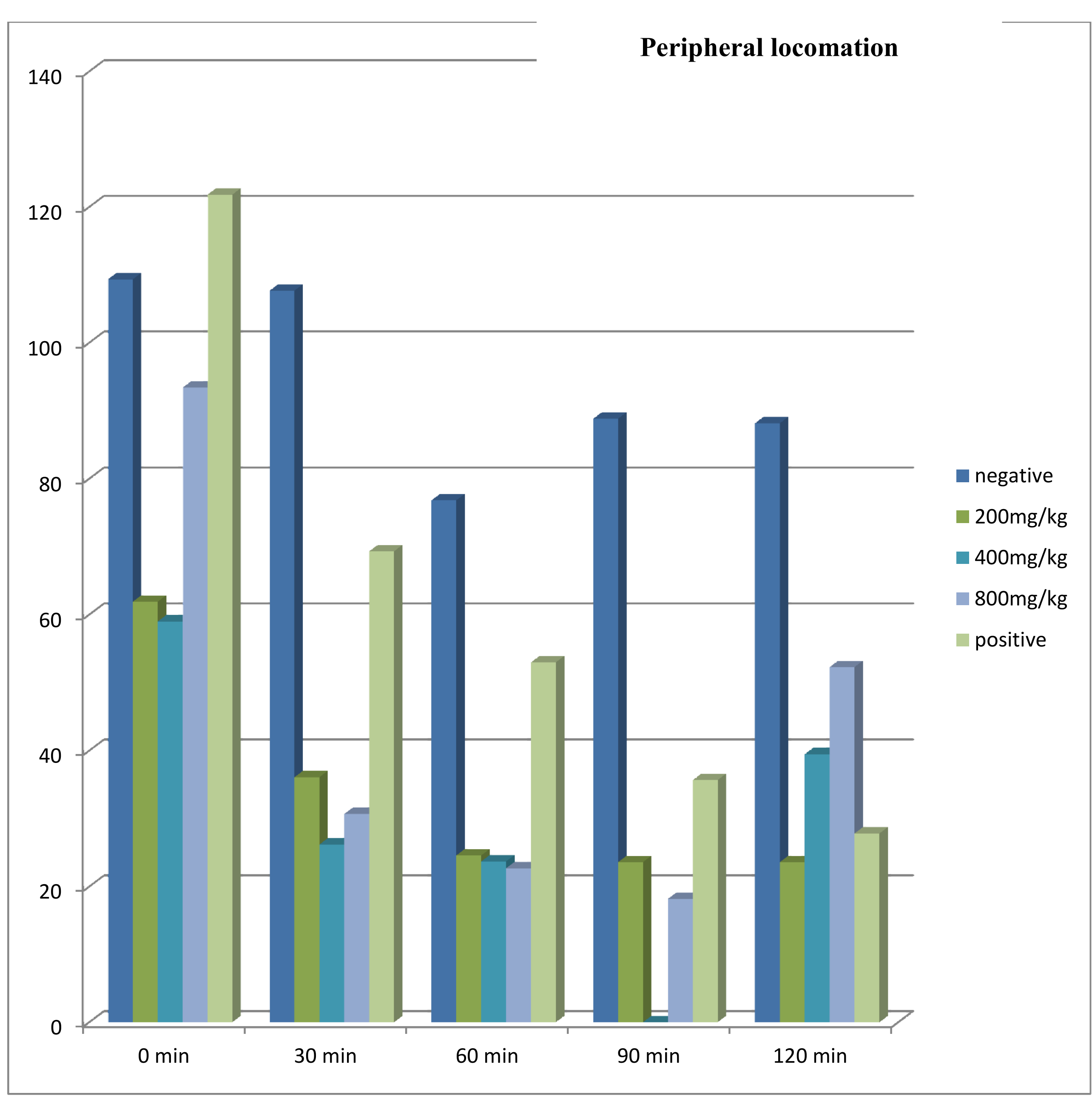


Figure 5.3: Graphical Presentation of CNS Activity of plant extract of *Grewia asiatica* by Open Field Test (Peripheral locomotion) in Mice.

Table 5.3: CNS Activity of plant extract of Grewia asiaticaby 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 Grewia asiatica	200mg/kg	4.700±0.81	3.000±.61	3.000±.683	3.000±0.61	3.000±0.61
Crude extract of Grewia asiatica	400mg/kg	2.9000±0.50	2.800±0.24	2.500±0.30	2.300±.33	3.700±0.57
Crude extract of Grewia asiatica	800mg/kg	5.400±0.74	3.2000±0.62	2.5000±0.47	2.400±.49	3.200±0.55

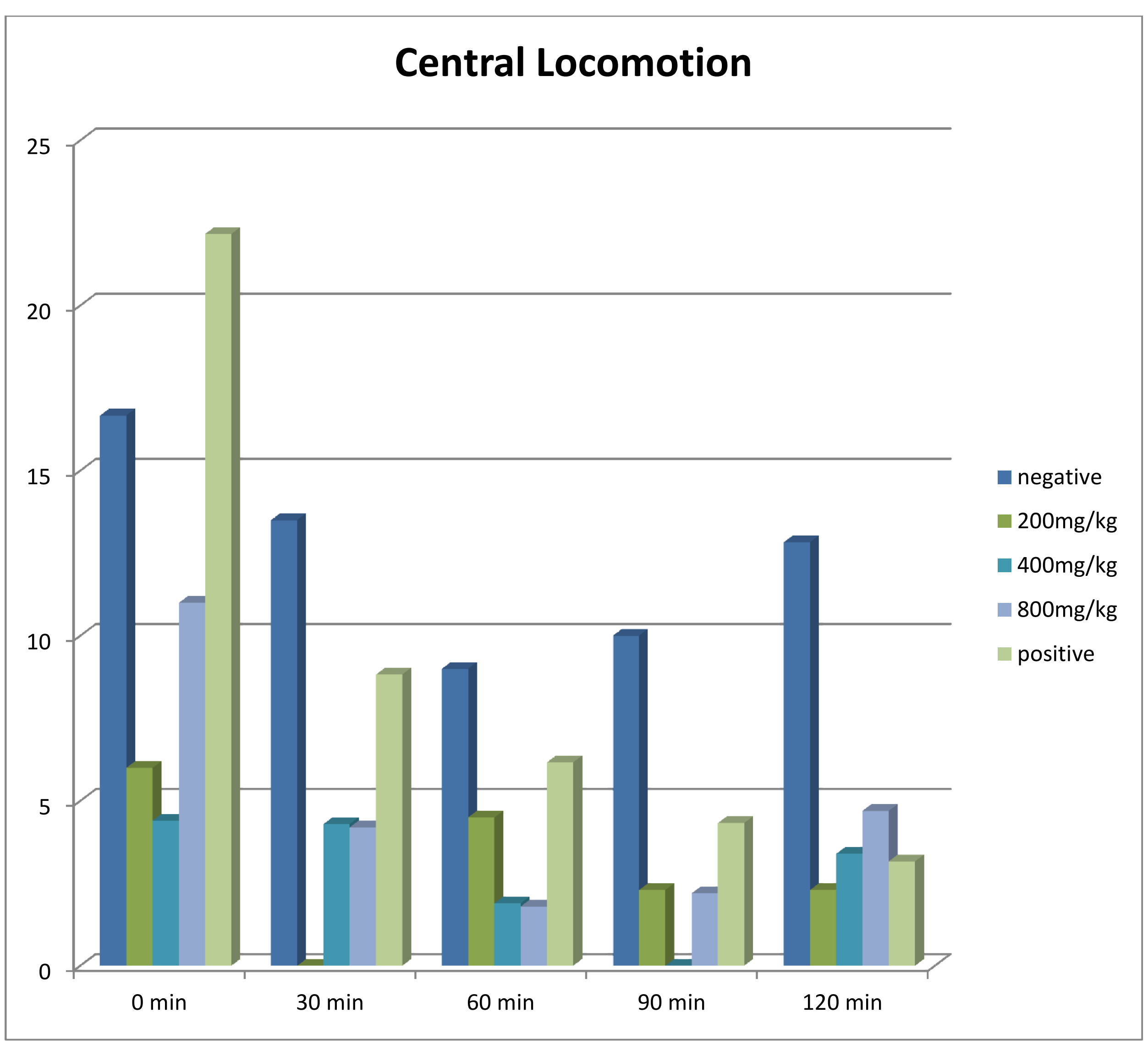


Figure 5.4: Graphical Presentation of CNS Activity of plant extract of *Grewia asiatica* by Open Field Test (Central Locomotion) in Mice.

Table 5.4: CNS Activity of plant extract of *Grewia asiatica*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 Grewia asiatica	200mg/kg	6.000±1.220	5.300±1.247	4.500±.73	2.300±.803	2.300±.803
Crude extract of Grewia asiatica	400mg/kg	4.400±.921	4.300±.6333	1.9000±.314 4	2.800±.489	3.400±.76303
.80Crude extract of Grewia asiatica	800mg/kg	11.000±.534	4.2000±.9977	1.800±.853	2.200±.663	4.700±.
Positive control, Diazepam	1mg/kg	22.17±1.08	8.83±0.31	6.17±0.31	4.33±0.33	3.17±0.48.869

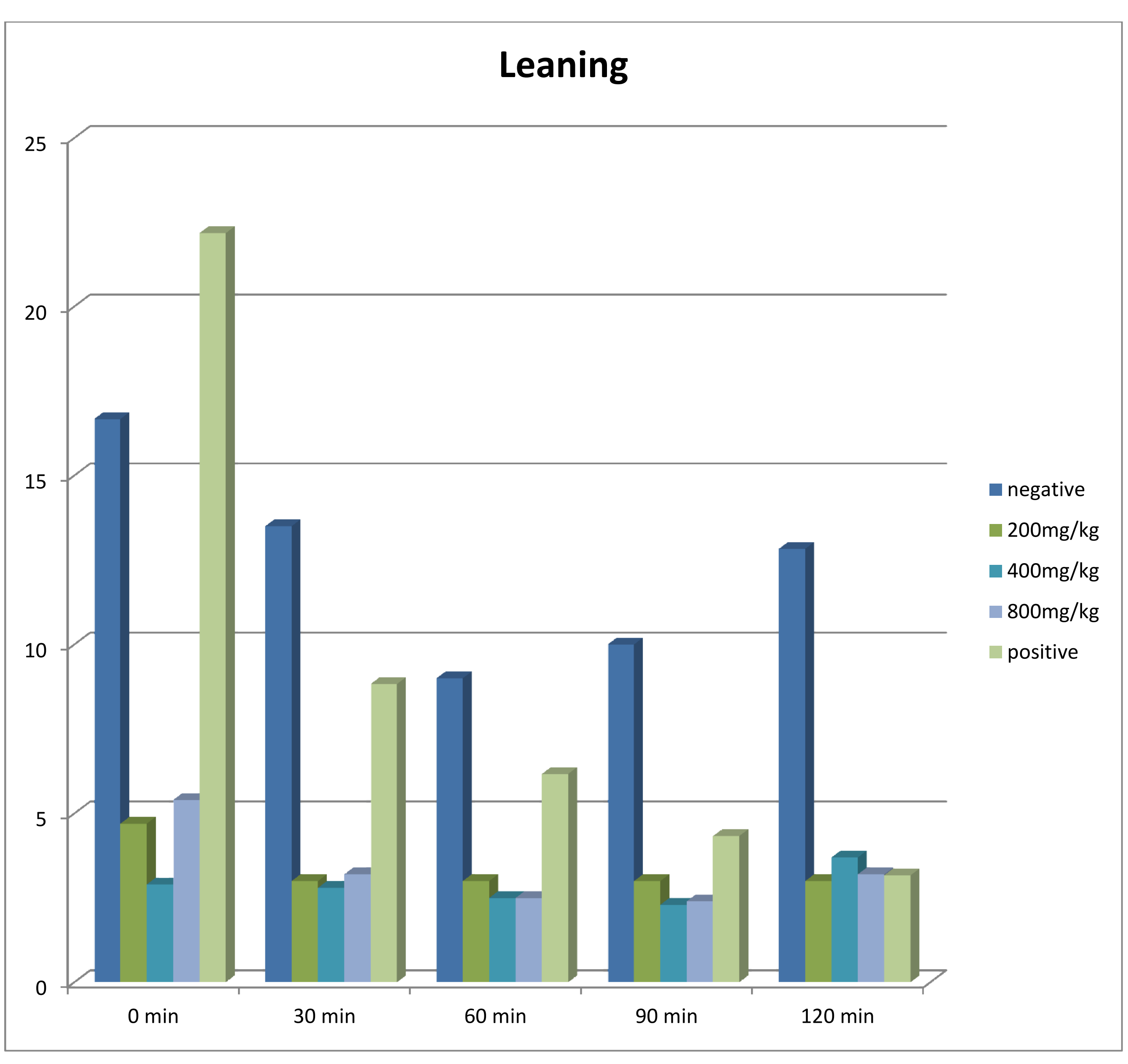


Figure 5.5: Graphical Presentation of CNS Activity of plant extract of *Grewia asiatica* by Open Field Test (Leaning) in Mice.

5.3. Acute and Chronic Toxicity Test:

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

5.3.2. Chronic Toxicity Test:

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

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

In the chronic study of methanolic extract of ***Grewia asiatica*** 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 ***Grewia asiatica*** 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***Grewia asiatica***

Table 5.6 : Effect of methanolic extract of *Grewia asiatica* 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
***Grewia asiatica*200mg/kg**	Female	19.1±0.525	33.1±1.86	1
***Grewia asiatica*200mg/kg**	male	19.1±0.525	34.70±2.70	2
***Grewia asiatica*400mg/kg**	Female	19.1±0.525	23.60±0.50	3
***Grewia asiatica*400mg/kg**	male	21.65±1.021	35.50±6.50	2
***Grewia asiatica*800mg/kg**	Female	21.80±1.138	37.93±2.90	3
***Grewia asiatica*800mg/kg**	male	22.30±1.23	32.6±0.00	3

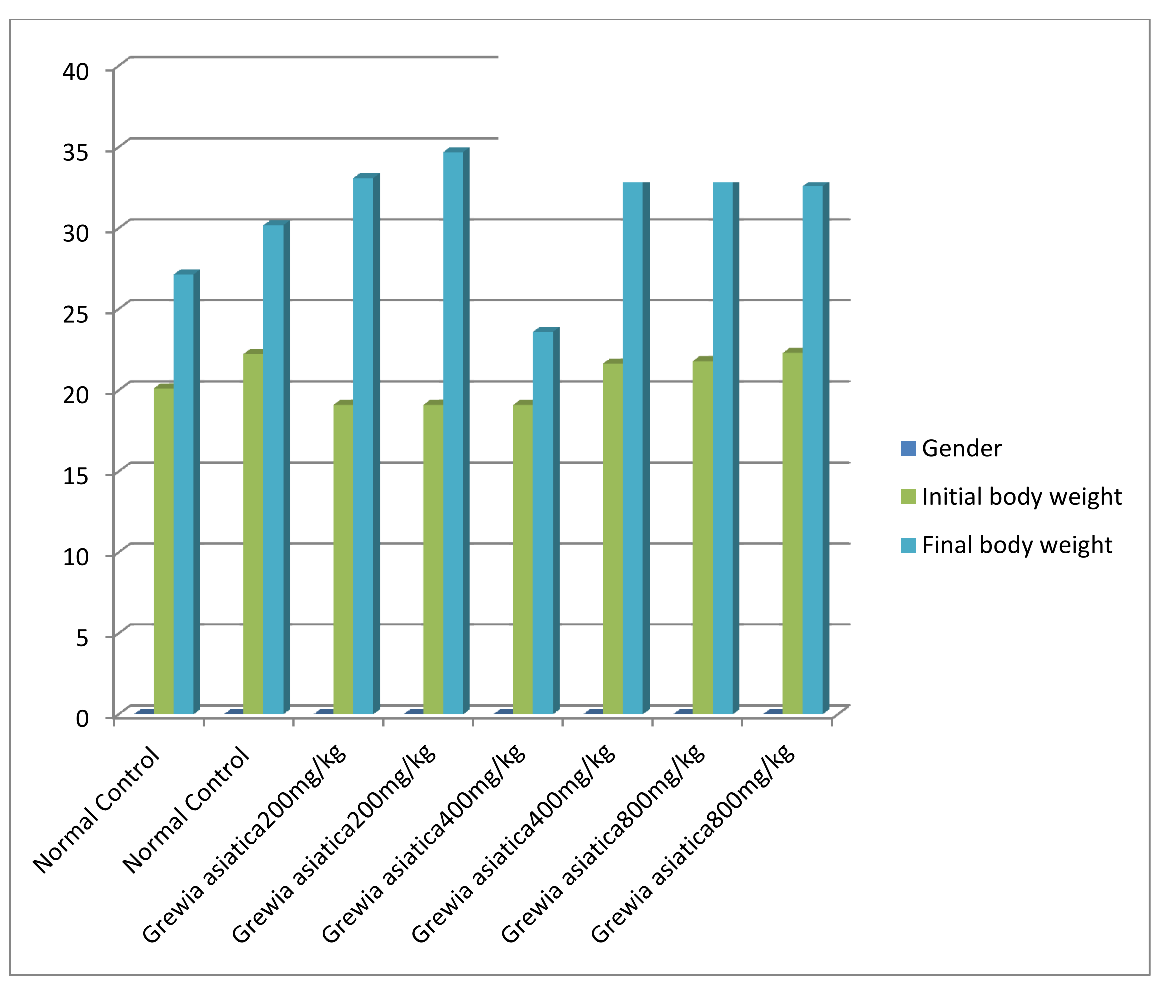


Figure 5.6: Graphical Presentation of Effect of methanolic extract of *Grewia asiatica* on body weight in mice

Table 5.7: Effect of *Grewia asiatica* 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
***Grewia asiatica*(200mg/kg, Female)**	6.19	18.2	82.5	2.7	0.6	0.2
***Grewia asiatica*(200mg/kg, male)**	2.3	26.9	72.2	3.5	0	5.2
***Grewia asiatica*(400mg/kg, Female)**	16.94	0	0	1.3	0.2	0
***Grewia asiatica* (400mg/kg, male)**	3.27	12.6	81	5.5	0.3	0.9
***Grewia asiatica* (800mg/kg,female)**	10.59	29.4	77.3	6.4	0.8	0.8
***Grewia asiatica* (800mg/kg, male)**	3.14	0	86.3	1	0	2.9

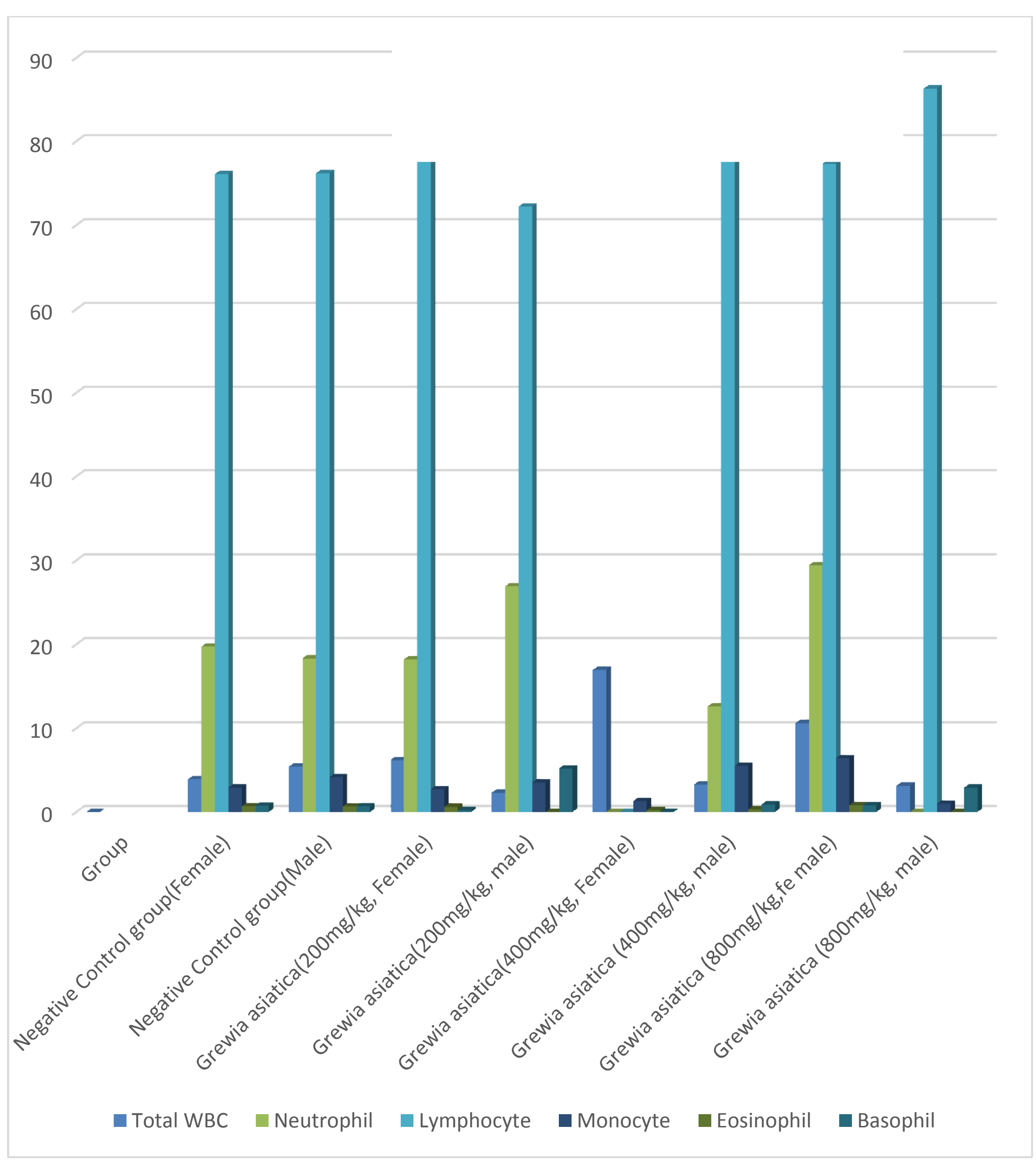


Figure 5.7 : Effect of *Grewia asiatica* on the Different count of WBC (White Blood Cell)

Table 5.8 : Effect of *Grewia asiatica* on the count of RBC (Red Blood Cell)

Group	Total RBC $10^6/mm^3$(n)	Haemoglobin	HCT	MCV	MCH	MCHC	RDW
Negative Control group(Female)	8.33	14.5	55.5	64.25	16.62	25.9	21.58
Negative Control group(Male)	8.79	13.55	50.3	56.7	15.28	26.93	22.28
***Grewia asiaticai* (200mg/kg, Female)**	7.92	12.3	36.4	46	15.5	33.8	10.8
***Grewia asiatica* (200mg/kg, male)**	8.24	11.9	37.2	45.1	14.4	32	14.1
***Grewia asiaticai* (400mg/kg female,)**	3.54	4.6	17.9	50.6	13	25.7	0
***Grewia asiaticai* (400mg/kg, Male)**	7.84	11.3	38.3	48.9	14.4	29.5	10.8
***Grewia asiaticai* (800mg/kg, female)**	9.37	13.5	42.8	45.7	14.4	31.5	16.5
***Grewia asiaticai* (800mg/kg, Male)**	8.19	12.5	36.8	44.9	15.3	34	11.2

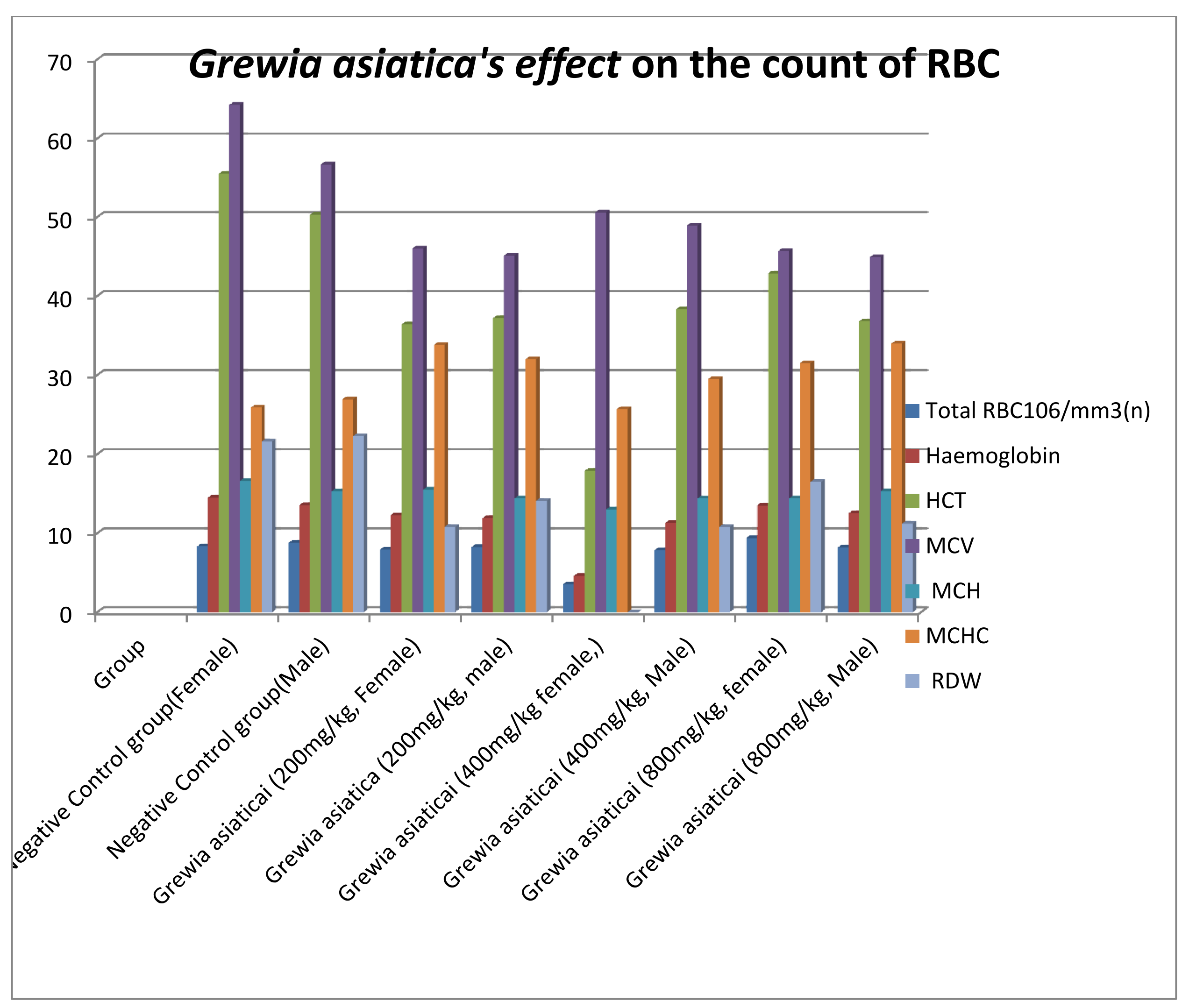


Figure 5.8: Effect of *Grewia asiatica* on the count of RBC (Red Blood Cell)

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

Treatment Group	Platelet
male (-)	**$10^3/mm^3$(n)**
Female (-)	848
GA200mg/kg (male)	895
GA400mg/kg (female)	820
GA400mg/kg (male)	820
GA400mg/kg (Female)	800
GA800mg/kg (male)	540
GA800mg/kg (Female)	514

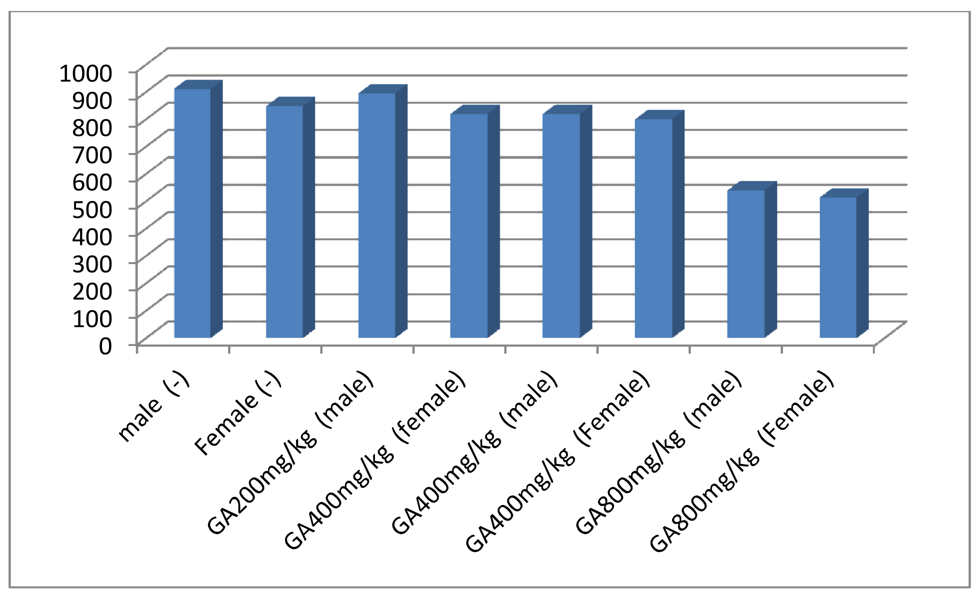


Figure 5.9:Effect of *Grewia asiatica*on Platelet on the CBC (Count Blood Cell) Test

Table 5.10: Effect of *Grewia asiatica*on the Liver Function Test

Treatment Group	SGPT (IU/dl)	SGOT (IU/dl)	SALP (IU/dl)
male (-)	65	31.83	141.5
female (-)	45	33.33	102.17
GA200mg/kg (male)	15	132	34
GA400mg/kg (female)	142	109	18
GA400mg/kg (male)	97	61	69
GA400mg/kg (female)	24	161	64
GA800mg/kg (male)	29	16	159
GA800mg/kg (female)	157	109	139

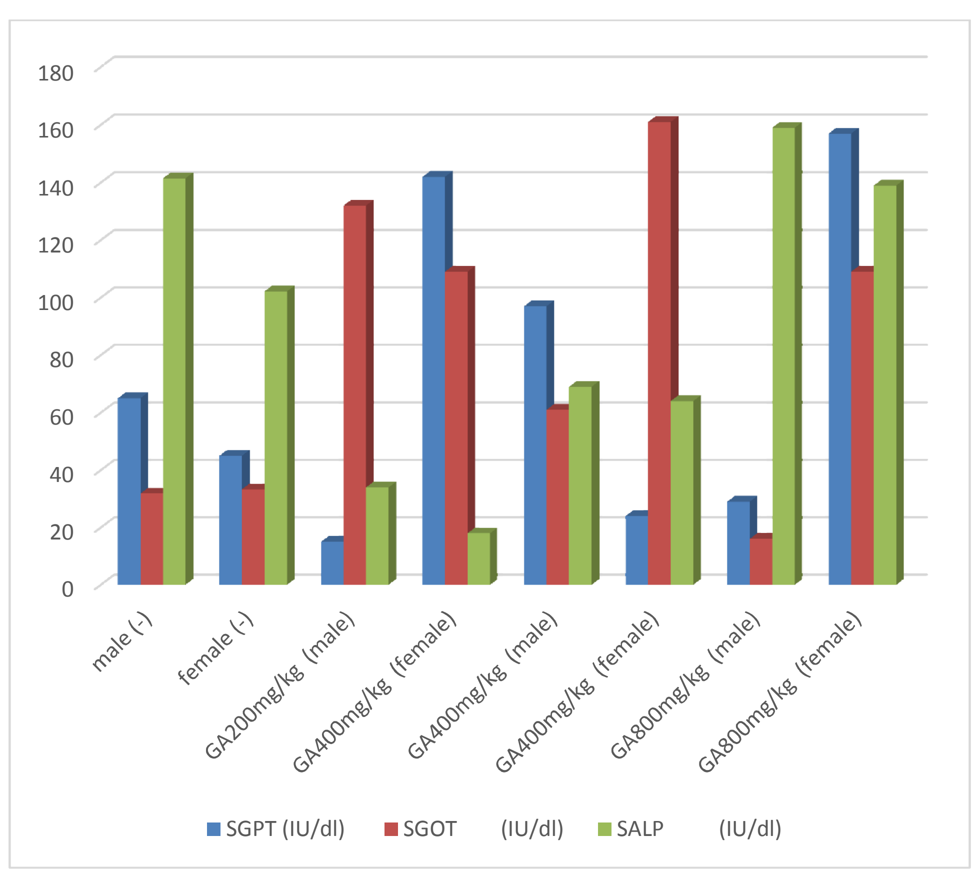


Figure 5.10: Effect of*Grewia asiatica*on the Liver Function Test

Grewia asiatica leaf					
Dose	Blood source	Code	Clot formation	clot	Clot lysis
1mg/ml	Person 1	GA1			
	Person 2	GA2			
5mg/ml	Person 1	GA3			
	Person 2	GA4			
10mg/ml	Person 1	GA5			

Thrombolytic activity:

Table5.11: percent clot lysis of Grewia asiatica

Dose	BLOOD SOURCE	NEGATIVE	GA LEAF	POSITIVE
1mg/ml	PERSON1	6.896552	24.69399	52.2523
	PERSON 2	8.057203	39.80293	50.2075
5mg/ml	PERSON1	8.432148	41.75824	56.5401
	PERSON2	8.15508	15.78298	49.8024
10mg/ml	PERSON1	12.40033	21.08234	52.5424
	PERSON1	11.77876	24.27912	56.993

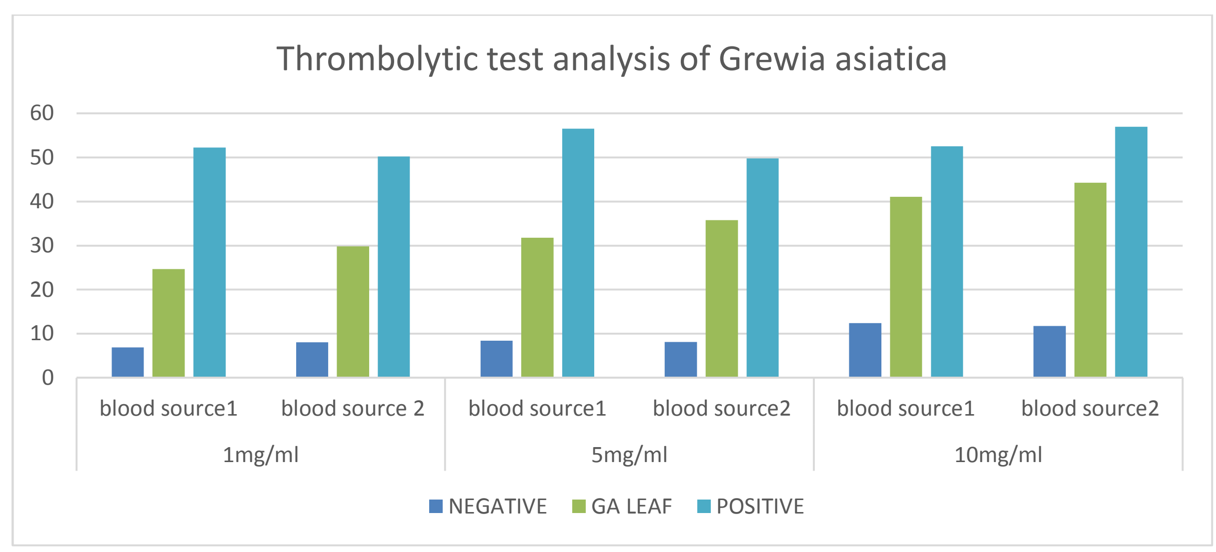


Fig5.11.A: percent clot lysis of Grewia asiatica

6.1 Total Phenolic Content Assay

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		

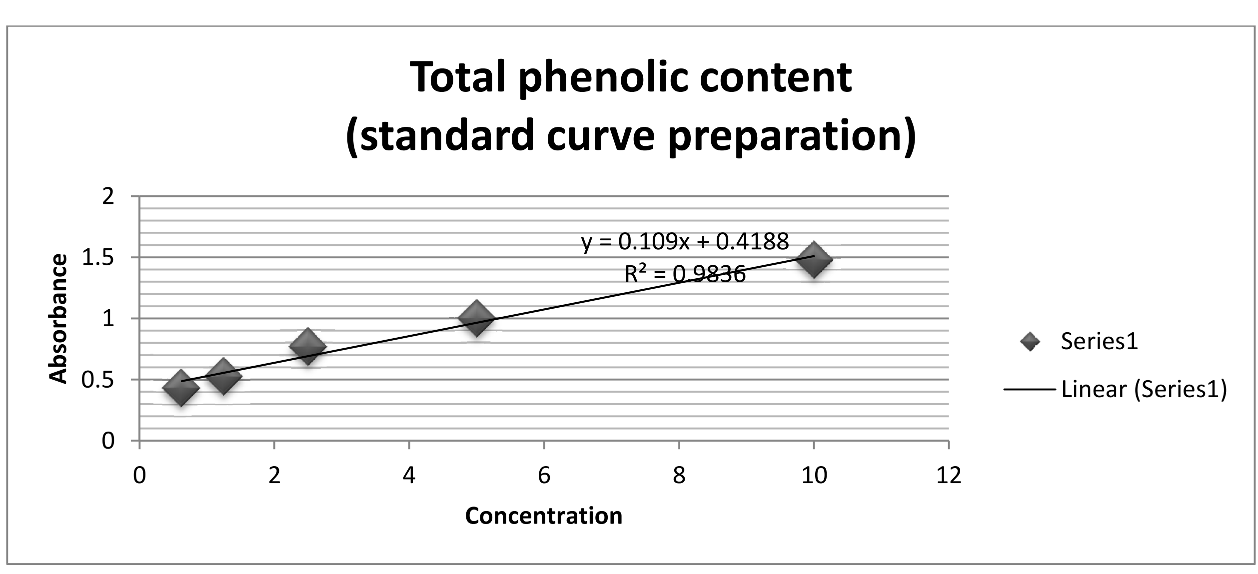


Fig 6.1.1.: Total Phenolic Content (Standard Curve)

Table 6.1.2: Total Phenolic Content of *Grewia asiatica*

Absorbance	mgSAE/g	Mean
0.868	41.28	54
1.090	61.65	
1.062	59.08	

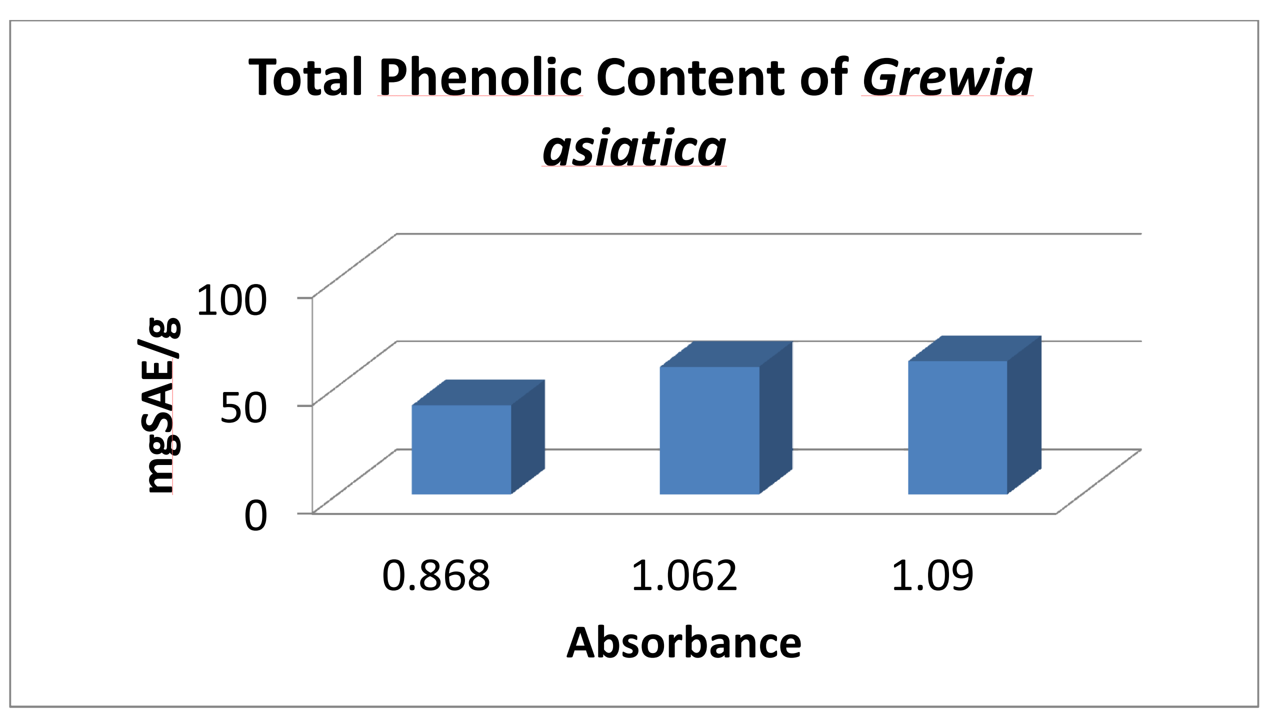


Table 6.2.1: DPPH Radical Scavenging Assay of Ascorbic Acid (Standard)

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	

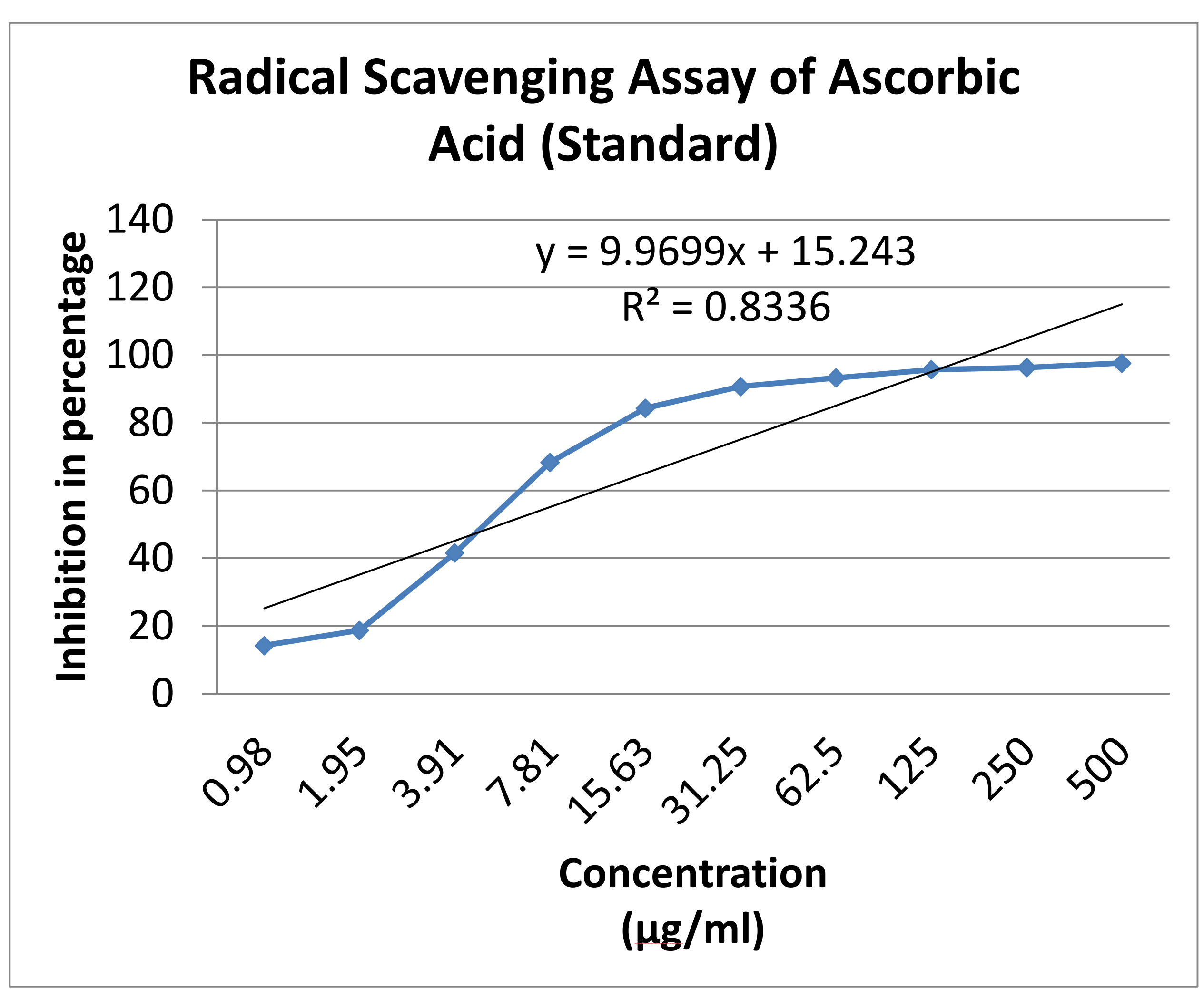


Table 6.2.5.: DPPH Radical Scavenging Assay of *Grewia asiatica*

Absorbance of Blank	Concentration µg/ml	Absorbance of the Sample	% of Inhibition	IC_{50} (µg/ml)
0.470	0.98	0.467	0.64	22.48
	1.95	0.455	3.19	
	3.91	0.447	4.89	
	7.81	0.431	8.29	
	15.63	0.406	13.61	
	31.25	0.361	23.19	
	62.5	0.307	34.68	
	125	0.212	54.89	
	250	0.062	86.80	
	500	0.032	93.19	

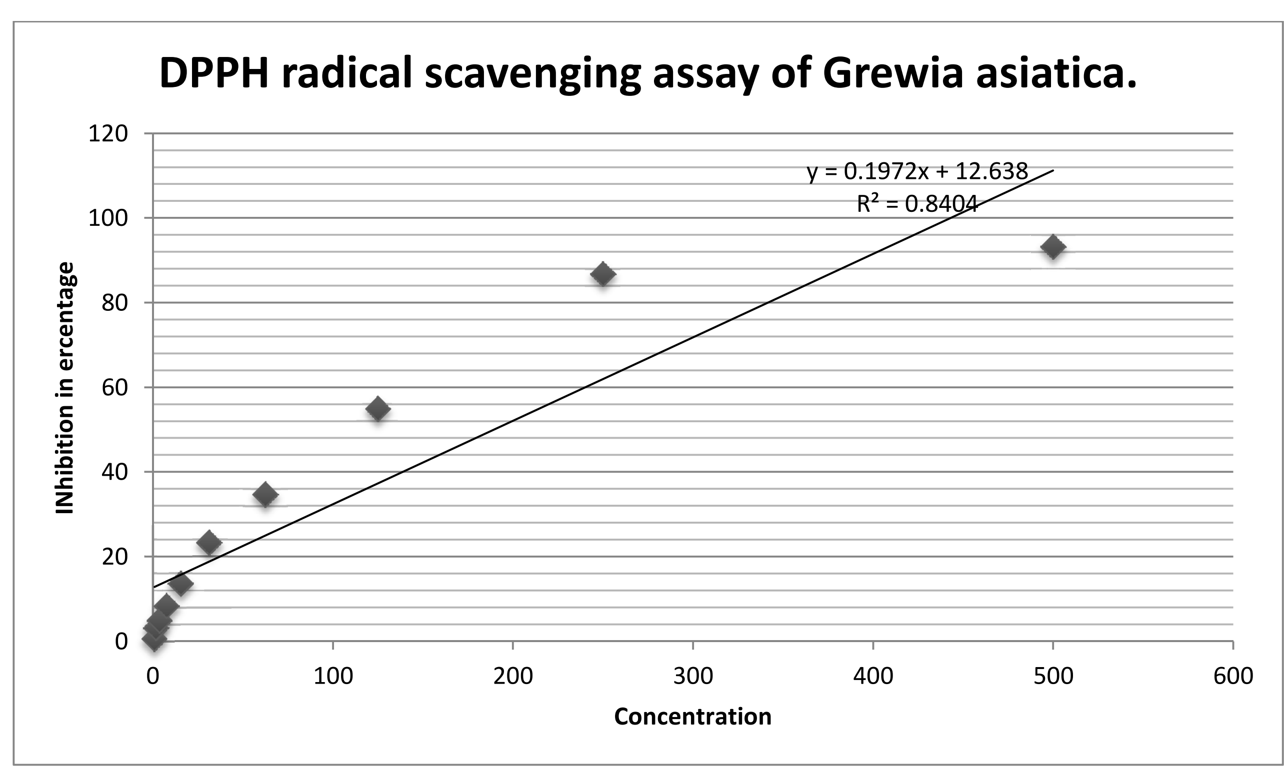


Fig 6.2.5: DPPH Radical Scavenging Assay of *Grewia asiatica*

6.4. Reducing Power Assay

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

Table 6.4.1Reducing Power Assay of Ascorbic acid

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

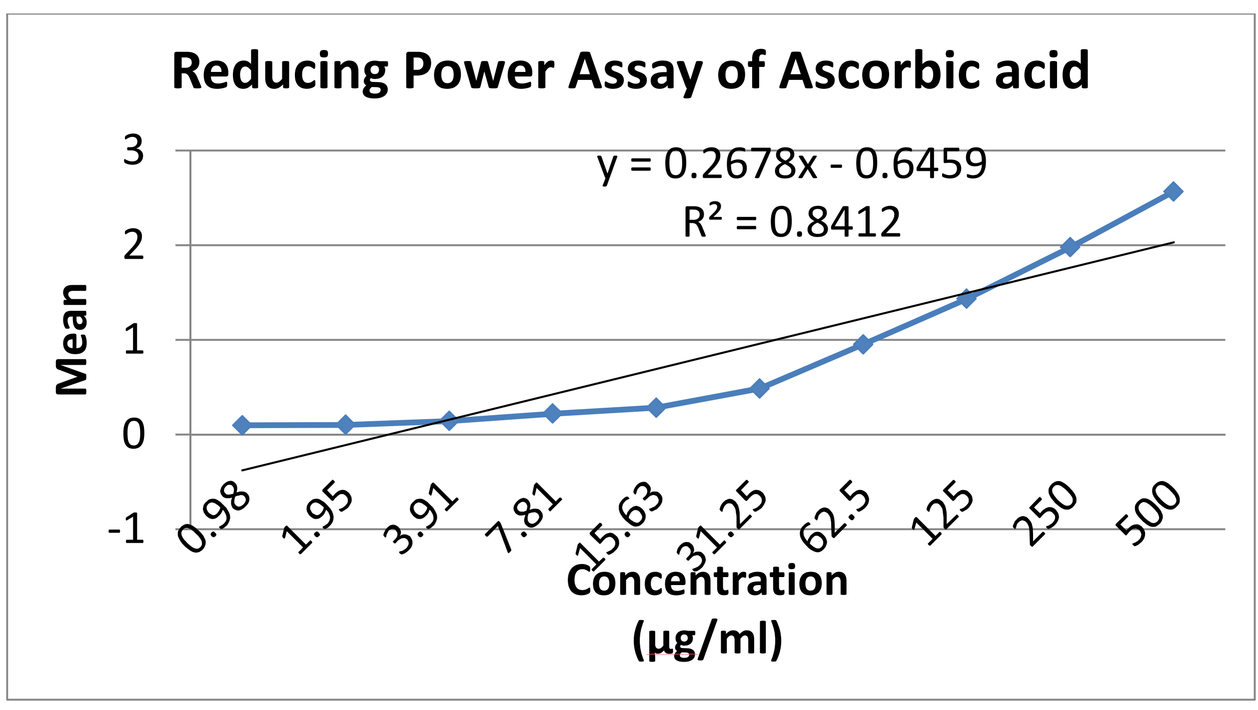


Table 6.4.5: Reducing Power Assay of *Grewia asiatica*

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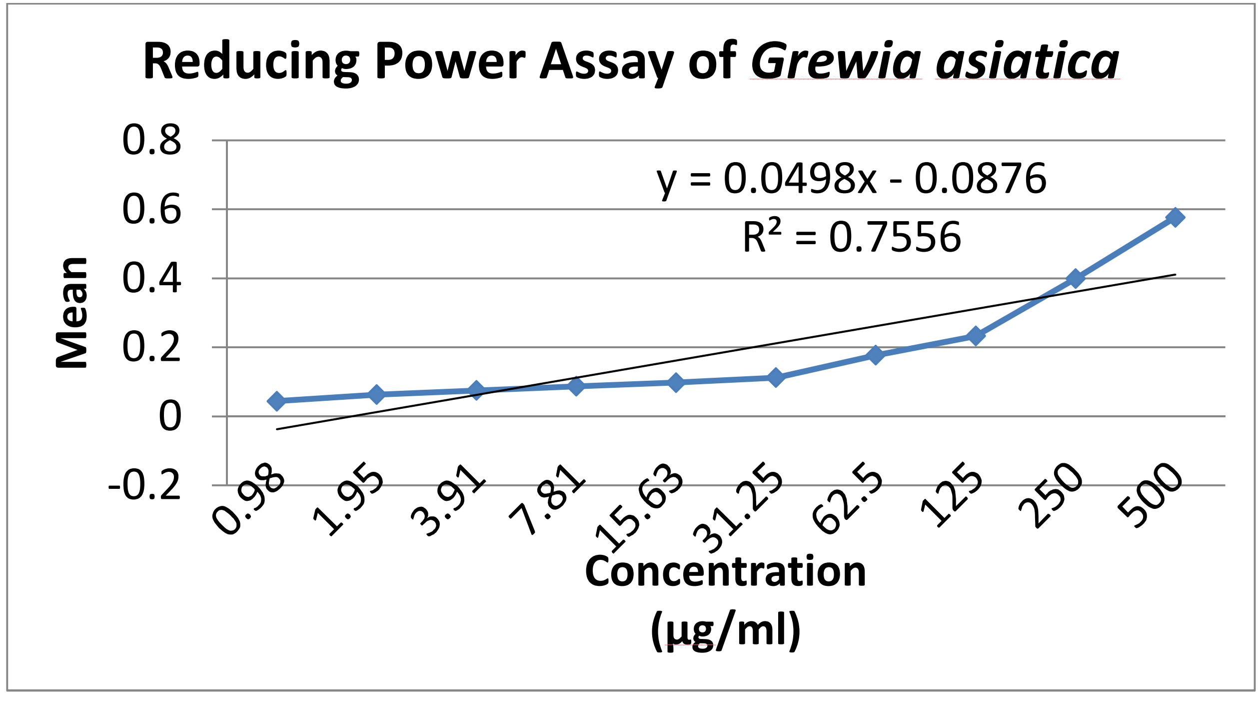
SL.	Concentration µg/ml	Absorbance			Mean
		1	2	3	
1	0.98	0..044	0.043	0.046	0.044
2	1.95	0.063	0.062	0.066	0.063
3	3.91	0.076	0.074	0.076	0.075
4	7.81	0.087	0.088	0.087	0.087
5	15.63	0.098	0.098	0.099	0.098
6	31.25	0.113	0.110	0.114	0.112
7	62.5	0.179	0.176	0.179	0.177
8	125	0.234	0.232	0.235	0.233
9	250	0.398	0.399	0.400	0.399
10	500	0.578	0.576	0.578	0.577



Chapter 6

Conclusion

6. Conclusion

Traditional medicines are mostly utilized by means of the natural products isolated from natural resources such as plant extracts. Pharmacological studies always reveal the potential medicinal properties of plants of our surroundings. Ethnobotanical data on the traditional uses of plants encourage the isolation of secondary metabolites leading to new lead compounds. With the increasing demands of inventing new drugs the pharmacological assay of natural plant resources play an 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, Acute and Chronic toxicity of methanolic extract of *Grewia asiatica*

This study shows that the prokinetic and laxative activities of extracts of) *Grewia asiatica* in mice are partially mediated through muscarinic receptors. Thus, this study provides sound mechanistic basis for the medicinal use of *Grewia asiatica* 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 *Grewia asiatica* (200mg/kg, 400mg/kg & 800mg/kg) dose dependently reduces the number of peripheral locomotion, central locomotion and leaning in the open field test. The reduction is significant (***$p<0.001$) when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Diazepam 1mg/kg. The effect of the extract is comparable to that of the standard drug, Diazepam 1mg/kg. The reference drug is found slightly potent than the extract.

The aim of the study was also to investigate the possible toxicity of the plant *Grewia asiatica* 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 *Grewia asiatica* 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 *Grewia asiatica* 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 *Grewia asiatica* exhibited Laxative activity, Depressant activity, and shows toxicity safety in acute and chronic toxicity studies in mice.

Chapter 7

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