Study of Pharmacological Activities of Methanolic Extract of *Stephania japonica*

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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, **Syeda Farjana Sarwar**, hereby declare that, **'Study of Pharmacological Activities of methanolic extract of *Stephania japonica*'**, submitted by me to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy (B.Pharm) is a confide record of original research work carried out by me under the supervision and guidance of **Dr.Shamsun Nahar Khan**, Associate professor and chairperson, Department of Pharmacy, East West University, Bangladesh. I also declare that no part of this report has been or is being submitted elsewhere for the award of any Degree.

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Certificate by the Supervisor

This is to certify that the dissertation entitle, **'Study of Pharmacological Activities of methanolic extract of *Stephania japonica*'**,submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the Degree of Bachelor of Pharmacy , was carried out by Syeda Farjana Sarwar ID No. 2012-1-70-033 under my supervision and no part of this dissertation has been or is being submitted elsewhere for the award of any Degree.

_____

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Endorsement by the Chairperson

This is to certify that the entitled **'Study of Pharmacological Activities of methanolic extract of *Stephania japonica*'**, is a genuine research work carried out by Syeda Farjana Sarwar, ID No. 2012-1-70-033 under the supervision of **Dr.Shamsun Nahar Khan**, Associate professor and chairperson, East West University, Dhaka. I further certify that no part of the thesis has been submitted for any other degree and all the resources of the information in this connection are duly acknowledged.

_____

Dr. Shamsun Nahar Khan

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Syeda Farjana Sarwar

DEDICATION

This research paper dedicated

To

My beloved parents

ABSTRACT

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

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

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

All data were analyzed by using SPSS analytical method.

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

Keywords: *Stephania japonica*, Neuropharmacological effect and Toxicity test.

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INTRODUCTION

1.1 Medicinal plant

A large portion of the World population, especially in developing countries, depends on traditional medicine for the treatment of diseases and injuries. Hundreds of the plant genera, to mention the most important natural resource of indigenous medicine, are used for that purpose, including very potent and powerful drugs which have stood the test of time and could not be replaced by modern medical preparations . The World Health Organization reported that 80 % of the world population rely chiefly on indigenous medicine and that the majority of traditional therapies involve the use of plant extracts or of their active constituents . In the recent years interest in herbal medicines has increased considerably both at home and abroad as they are believed to be comparatively less toxic than the synthetics . (Mahbubur et al, 2013) It is estimated that 70– 80% of people worldwide rely chiefly on traditional, largely herbal medicine to meet their primary healthcare needs. For cardiovascular diseases plant or herbal treatments have been used in patients with congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis,anticancer activity, cerebral insufficiency and venous insufficiency. It has also been observed that a number modern drugs has been derived from plants used by the indigenous people. (Kabidul et al,2014)

1.1.1Definition of medicinal plant

Medicinal plants are various plants used in herbalism and thought by some to have medicinal properties. The definition of Medicinal Plant has been formulated by WHO (World Health Organization) as follows- "A medicinal plant is any plant which, in one or more of its organ, contains substance that can be used for therapeutic purpose or which is a precursor for synthesis of useful drugs." (Mohammed,2013)

When a plant is designated as 'medicinal', it is implied that the 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".Many of the plants could be used as stimulants, poisons, hallucinogens or as medicine because of the presence of unique or rich biological-active plant chemicals (i.e. Chemical compounds that have a biological effect on another organism (Hamburger& Hostettmann, 1991).

1.1.2 Importance of medicinal plant

• Plants are directly used as medicines by a majority of cultures around the world, for example Chinese medicine and Indian medicine.Many food crops have medicinal effects, for example garlic.About 25% of the drugs prescribed worldwide come from plants, 121 such active compounds being in current use for examples of 252 of important drugs obtained from plants are digoxin from *Digitalis* spp., quinine and quinidine from *Cinchona* spp., vincristrine and vinblastine from *Catharanthus roseus*, atropine from *Atropa belladonna* and morphine and codeine from *Papaver somniferum*. It is estimated that 60% of anti-tumour and anti-infectious drugs already on the market or under clinical trial are of natural origin. (Yue-Zhong Shu, 1998)

Medicinal plants have many characteristics when used as a treatment, as follow:

a) Synergic medicine- The plants ingredients all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.
b) Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.
c) Preventive medicine- It has been proven that the component of the plants are also characterized by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effect of synthetic treatment (Ghani, 1998).

❖ Plants are valuable for modern medicine in four basic ways:

a) They are used as sources of direct therapeutic agents.
b) They serve as raw materials base for elaboration of more complex semi synthetic chemical compounds.
c) The chemical structures derived from plant sources can be used as models for new synthetic compounds.
d) Finally plants can be used as taxonomic markers for the discovery of new compounds. (Reddy, *et al.*2010).

1.1.3 Medicinal plants & Traditional Medicine Practice in Bangladesh

Bangladesh possesses arich 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. 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. Use of herbal medicines is thus, widespread in the world and in Bangladesh, but still very little is known about these medicinal plants.

Plants were once a primary source of all the medicines in the world and they still continue to provide mankind with remedies. Examples include "Quinine" obtained from *Cinchona spp* and used against malaria (antiprotozoal); "Taxol" from *Taxus brevifolius*,used as anticancer and "Atropine" from *Datura* spp, used as mydriatic and antispasmodic. Traditional medical practice among the tribal people is mainly based on the use of plant and animal parts and their various products as items of medicine. The medicaments, prepared from plant materials and other natural products sometimes also include some objectionable substances of animal origin. They are dispensed in a number of dosage forms like infusions, decoctions, pastes, moulded lumps, powders, dried pills, creams and poultices.(Ghani,1999)

1.1.4 Causes of toxicity with herbal products

Many plant products are toxic. In an herbal supplement, one product may produce desired therapeutic effect and other ingredients may produce toxicity, since all compounds are extracted together in making herbal supplements and good products are not usually separated from bad products, toxicity may occur of common herbal supplements. some herbal supplements may because toxicity is the dose size.From past few decades, plants make an important contribution to healthcare. Herbal preparations contain complex mixtures of one or more plants which contain active ingredients, plant materials in crude or processed form. The data existing for most plants to assurance their quality, efficacy and

safety is unacceptable. Majority of people who use herbal medicines do not inform their physician about their use. Herbal medicines can alter physiology and these changes can be reflected in irregular test results. There are some scientific evidences are there, which proves the herbal medicines can cause considerable toxicities. So proper control on the herbal supplements is needed.For example of Kava is an herbal sedative anti-anxiety or calming effect, and to relieve symptoms of throat pain as it produces a "numbing" effect on the tongue and throat. Toxicity Kava can have additive effects with central nervous system depressants. A patient who was taking alprazolam, cimetidine and terazosin became lethargic and disoriented after ingesting kava. Kava lactones can inhibit cytochrome P-450 activities and have a potential interaction with the drugs that are metabolized by the liver. Heavy consumption of kava has been associated with increased –gultamyltranferase.(Srivalli et al,2011)

1.1.5 Prevalence of toxicity with herbal products

Herbal medicines are in great demand in both developed and developing countries as a source of primary health care owing to their attributes having wide biological and medicinal activities, high safety margins and lesser costs. Herbal molecules are safe and would overcome the resistance produced by the pathogens as they exist in a combined form or in a pooled form of more than one molecule in the protoplasm of the plant cell (Lai and Roy, 2004; Tapsell et al., 2006). Even with the advent of modern or allopathic medicine, Balick and Cox (1996) have noted that a number of important modern drugs have been derived from plants used by indigenous people.

Current research in drug discovery from medicinal plants involves a multifaceted approach combining botanical, phytochemical, biological, and molecular techniques. Medicinal plant drug discovery continues to provide new and important leads against various pharmacological targets including cancer, HIV/AIDS, Alzheimer's, malaria, and pain. Several natural product drugs of plant origin have either recently been introduced to the United States market, including arteether, galantamine, nitisinone, and tiotropium, or are currently involved in late-phase clinical trials. As part of National Cooperative Drug Discovery Group (NCDDG) research project, numerous compounds from tropical rainforest plant species with potential anticancer activity have been identified.. (Marcy,2005)

1.2. 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.

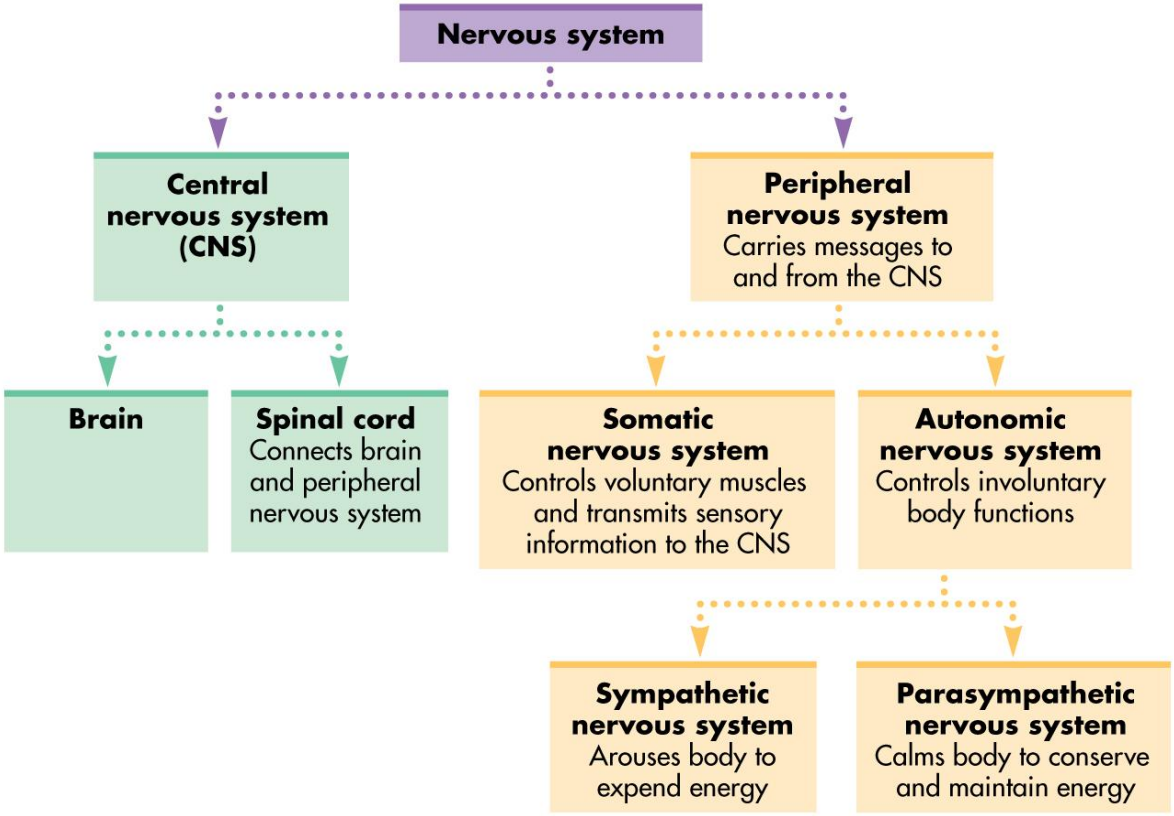


Figure1.1: Organization of the Human Nervous System.

1.2.1. The central Nervous System

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

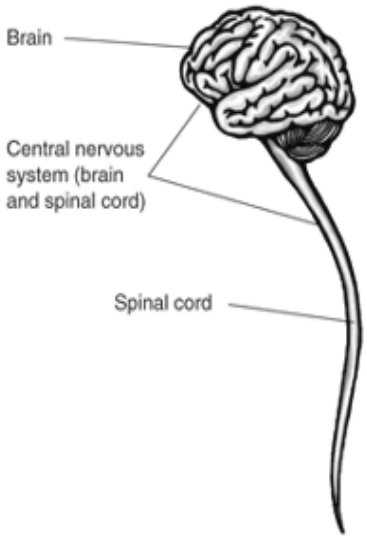


Figure1.2: Central Nervous System

1.2.1.1. Parts of Central Nervous System

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

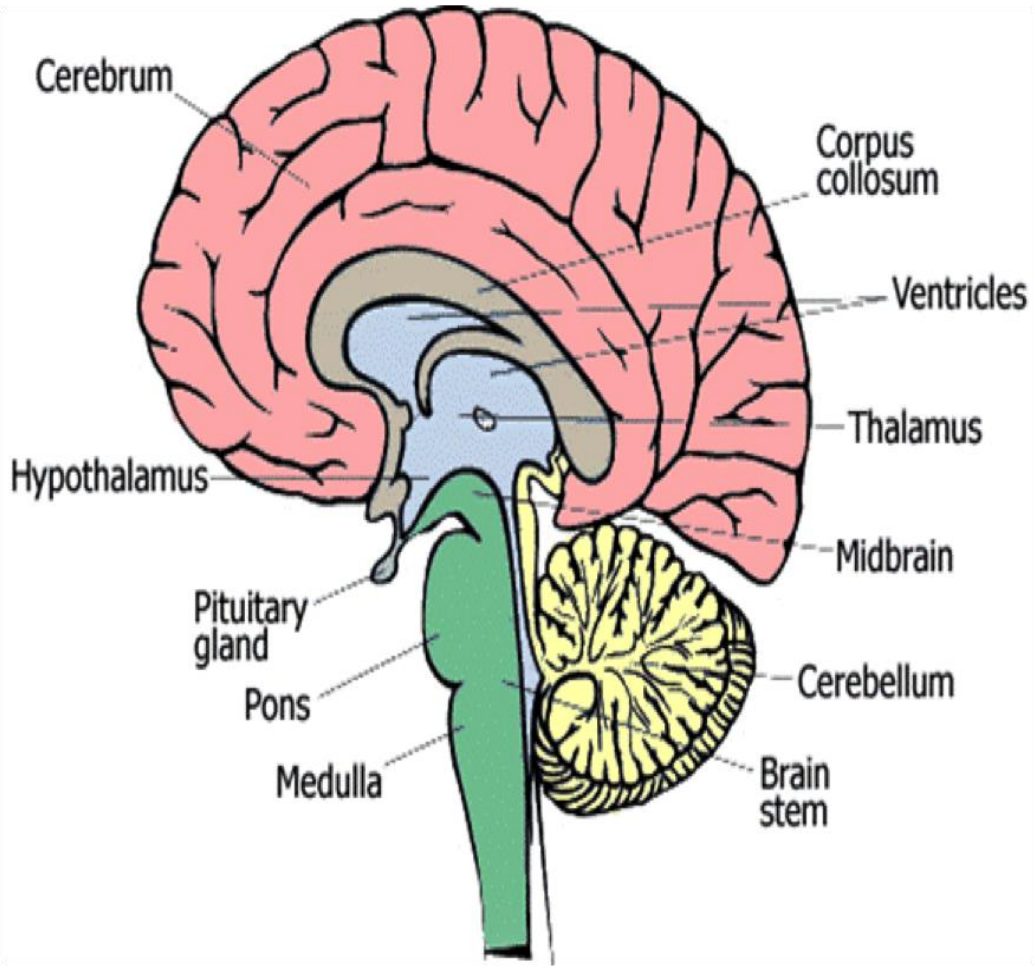


Figure1.3: Human Brain

1.2.2. Peripheral Nervous System:

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

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

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

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

1.2.3. 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.

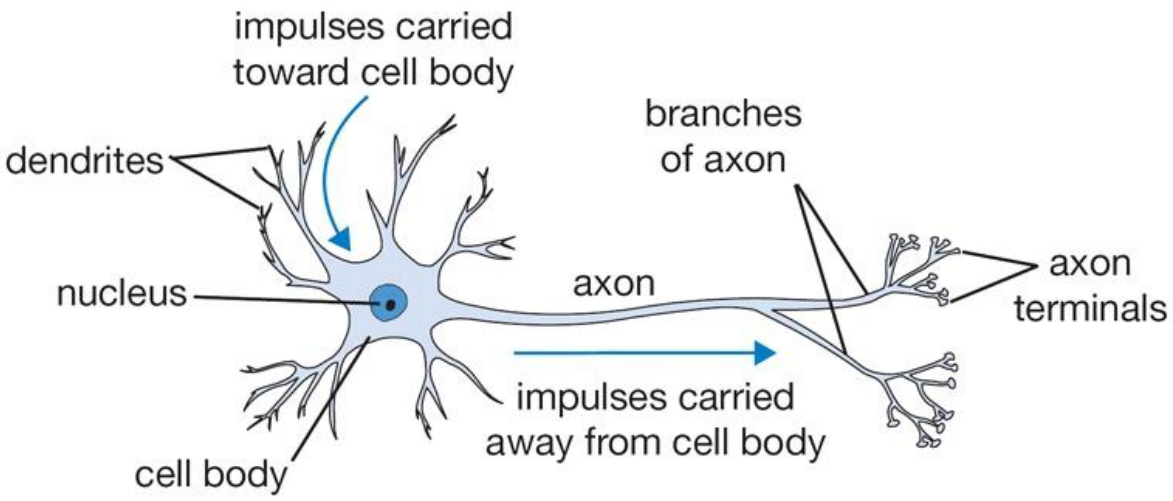


Figure1.4: Neuron

Cell Body or Soma:

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

Axon:

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

Dendrites:

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

1.2.4. Synapse

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

1.2.5. Different Central Nervous System Disorders

- ✓ **Alzheimer's disease**-A progressive, degenerative disease that occurs in the brain and results in impaired memory, thinking, and behavior.
- ✓ **Bradykinesia**- Slowness of movement.
- ✓ **Bradyphrenia**-Slowness of thought processes
- ✓ **Cerebral embolism**- A brain attack that occurs when a wandering clots (embolus) or some other particle forms in a blood vessel away from the brain - usually in the heart.
- ✓ **Cerebral hemorrhage**- A type of stroke occurs when a defective artery in the brain bursts, flooding the surrounding tissue with blood.

- ✓ **Cerebral thrombosis**- The most common type of brain attack; occurs when a blood clot (thrombus) forms and blocks blood flow in an artery bringing blood to part of the brain.
- ✓ **Delusions**- A condition in which the patient has lost touch with reality and experiences hallucinations and misperceptions.
- ✓ **Dementia**– It is not a disease itself, but group of symptoms that characterize diseases and conditions; it is commonly defined as a decline in intellectual functioning that is severe enough to interfere with the ability to perform routine activities.
- ✓ **Epilepsy** (Also called seizure disorder)-A brain disorder involving recurrent seizures.
- ✓ **Euphoria**– A feeling of well-being or elation; may be drug-related.
- ✓ **Guillain-Barré syndrome**- A disorder in which the body's immune system attacks part of the nervous system.
- ✓ **Headache (primary)**-Includes tension (muscular contraction), vascular (migraine), and cluster headaches not caused by other underlying medical conditions.
- ✓ **Headache (secondary)**-Includes headaches that result from other medical conditions. These may also be referred to as traction headaches or inflammatory headaches.
- ✓ **Meningitis**-An inflammation of the meninges, the membranes that cover the brain
- ✓ **Multiple sclerosis (MS)**-A disease of the central nervous system that is an unpredictable condition that can be relatively benign, disabling, or devastating, leaving the patient unable to speak, walk, or write.
- ✓ **Parkinson's disease (PD)**-The most common form of parkinsonism; a slowly progressing, degenerative disease that is usually associated with the following symptoms, all of which result from the loss of dopamine-producing brain cells: tremor or trembling of the arms, jaw, legs, and face; stiffness or rigidity of the limbs and trunk; bradykinesia (slowness of movement); postural instability, or impaired balance and coordination.
- ✓ **Seizure**- Occurs when part(s) of the brain receives a burst of abnormal electrical signals that temporarily interrupts normal electrical brain function. **(**Howland and Mycek, 2006).

1.3. 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).

1.3.1. Acute toxicity

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

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

1.3.2. Chronic toxicity

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

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

1.3.3. Toxic effects

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

In all the cases, the toxic effects are usually manifested either in an acute or a chronic manner, and occur mostly as a result of an acute or chronic exposure to toxic compound by oral ingestion, inhalation or absorption following skin contact the toxic effects are seen as (1) signs or reflection of a disturbance of the normal activities of enzymes that perform essential biochemical roles in all forms of life; (2) alteration of the normal activities of plasma membrane that regulate the exchange of nutrients and metabolites between the cell and its surroundings and (3) the disturbances of other

normal cell activities, e.g. RNA and DNA synthesis, growth, division and general metabolism at all levels of organization from sub-cellular to organ and organ system (Pascoe, 1983; Timbrell, 2002).

The way in which the toxic agent is introduced into the body also plays significant role.

1.3.3.1. 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).

1.3.3.1.1. Intra-peritoneal injection

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

1.3.3.1.2. Oral administration

The oral route is probably one of the most common means by which a chemical enters the body. Oral administration of chemicals that are rapidly absorbed from the gastrointestinal tract would theoretically expose the liver to concentrations of the agent that would not be obtained if other routes of administration were used (Loomis and Hayes, 1996). Furthermore, if a compound entered the enterohepatic cycle, at least a portion of the compound would be localized in the organs involved in the cycle .Compounds that are known to be toxic to the liver would be expected to be more toxic following oral administration on repeated occasions, whereas their administration by other routes may be less hazardous (Loomis and Hayes, 1996; Waynforth, 1980).

1.3.4 Cancer:

Cancer is the name given to a collection of related diseases. In all types of cancer, some of the body's cells begin to divide without stopping and spread into surrounding tissues.

Cancer can start almost anywhere in the human body, which is made up of trillions of cells. Normally, human cells grow and divide to form new cells as the body needs them. When cells grow old or become damaged, they die, and new cells take their place.

When cancer develops, however, this orderly process breaks down. As cells become more and more abnormal, old or damaged cells survive when they should die, and new cells form when they are not needed. These extra cells can divide without stopping and may form growths called tumors.

Many cancers form solid tumors, which are masses of tissue. Cancers of the blood, such as leukemias, generally do not form solid tumors.Cancerous tumors are malignant, which means they can spread into, or invade, nearby tissues. In addition, as these tumors grow, some cancer cells can break off and travel to distant places in the body through the blood or the lymph system and form new tumors far from the original tumor.

Unlike malignant tumors, benign tumors do not spread into, or invade, nearby tissues. Benign tumors can sometimes be quite large, however. When removed, they usually don't grow back, whereas malignant tumors sometimes do. Unlike most benign tumors elsewhere in the body, benign brain tumors can be life threatening. (National cancer institute,2006)

1.3.5.1 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.

1.3.5.2 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.

1.3.5.3 History of Cell counting

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

1.3.6 Cellular Elements of Blood

Blood is a circulating tissue composed of fluid plasma and cells (red blood cells, white blood cells, platelets). Anatomically, blood is considered a connective tissue, due to its origin in the bones and its function. Blood is the means and transport system of the body used in carrying elements (e.g. nutrition, waste, heat) from one location in the body to another, by way of blood vessels.

Blood is made of two parts:

1. Plasma which makes up 55% of blood volume.

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

1.3.6.1. Plasma

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

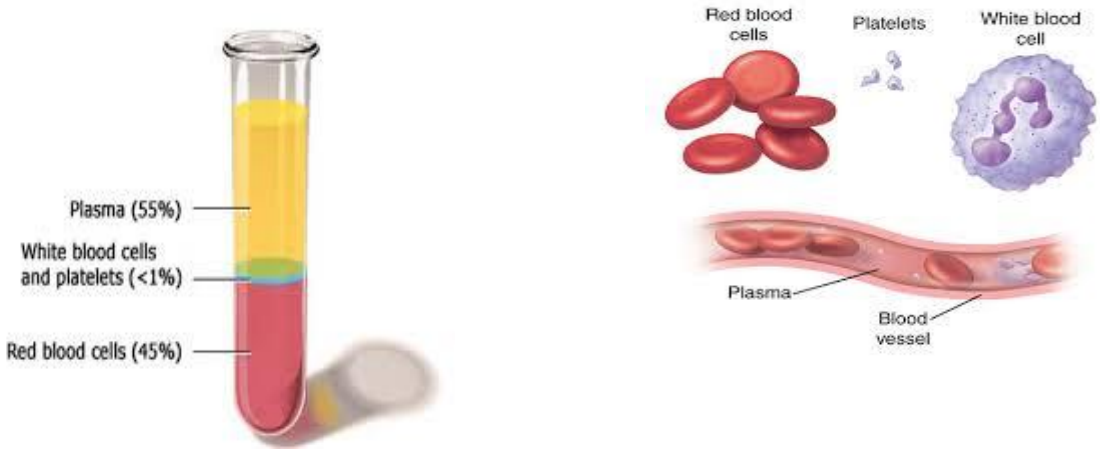


Figure1.5: Plasma of the Blood

1.3.7 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 1.6*:* Red Blood cell

- **Normal range of RBC 8-16×$10^6$$mm^3$**

1.3.7.1 Different count of RBC

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

Role in disease

- Hemoglobin deficiency can be caused either by decreased amount of hemoglobin molecules, as in anemia(Anemia is a decrease in number of red blood cells (RBCs) or less than the normal quantity of hemoglobin in the blood), or by decreased ability of each molecule to bind oxygen at the same partial pressure of oxygen.
- hemoglobin deficiency decreases blood oxygen-carrying capacity
- Other common causes of low hemoglobin include loss of blood, nutritional deficiency, bone marrow problems, chemotherapy, kidney failure, or abnormal hemoglobin
- High hemoglobin levels may be caused by exposure to high altitudes, smoking, dehydration, or tumors
- Elevated levels of hemoglobin are associated with increased numbers or sizes of red blood cells, called polycythemia.(Polycythemia is a disease state in which the proportion of blood volume that is occupied by red blood cells increases. Blood volume proportions can be

measured as hematocrit level. It can be due to an increase in the number of red blood cells or to a decrease in the volume of plasma . Polycythemia is sometimes called erythrocytosis)

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

Higher than Normal Hematocrit

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

Lower than Normal Hematocrit

- Infants without adequate iron intake
- children going through a rapid growth spurt, during which the iron available cannot keep up with the demands for a growing red cell mass.
- menstruating women, who have a greater need for iron because of blood loss during menstruation
- pregnant women, in whom the growing fetus creates a high demand for iron

- Patients with chronic kidney disease whose kidneys no longer secrete sufficient levels of the hormone erythropoietin that promotes RBC proliferation. Erythropoietin prevents the death of cells in the erythrocyte cell line in the bone marrow. Therefore, erythropoietin allows those cells to continue to mature, exit the bone marrow and become RBCs (Jelkmann, 2004).

Mean corpuscular volume, or **mean cell volume** (**MCV**)

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

Higher than Normal MCV

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

Lower than Normal MCV

- The most common causes of microcytic anemia are iron deficiency (due to inadequate dietary intake, gastrointestinal blood loss, or menstrual blood loss), thalassemia, sideroblastic anemia or chronic disease. In iron deficiency anemia (microcytic anemia), it can be as low as 60 to 70 femtolitres.
- In some cases of thalassemia, the MCV may be low even though the patient is not iron deficient (Tonnesen, 1986).

Mean corpuscular hemoglobin (MCH)

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

Higher than Normal MCH

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

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

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.
- .

1.3.8. 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

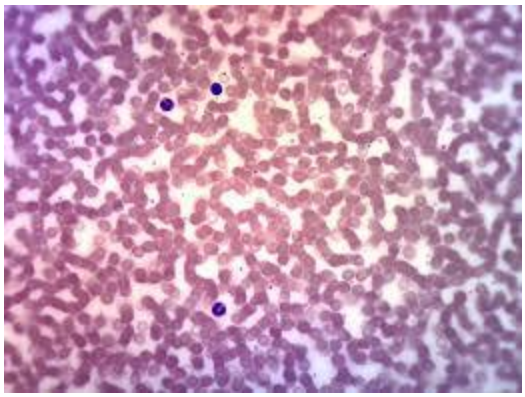


Figure 1.7: White Blood Cells

- **Normal range of WBC: 3-7×$10^3$$mm^3$**

Different count of WBC

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

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

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

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

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

1.3.9.Platelets (Thrombocytes):

Platelets are fragments of cytoplasm that are fired out in the blood from large cells in the bone narrow. So some physicians don't consider them complete blood cells. Platelets work importantly in blood clotting known as haemostasis. Vessel walls are surrounded by platelets to stop bleeding when injured. They also help in infections from enzymatic reactions. Normal range of platelet: 1000-1600×103mm3 (Ganong, 2003)

- **Normal range of platelet: 1000-1600×$10^3$$mm^3$**

1.3.10 Hepatotoxicity

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

Several factors predispose the liver to xenobiotic toxicity.

- Firstly, for chemicals entering the body via the oral route, anatomical proximity to the GI-tract ensures the liver is the 'first port of call' for ingested xenobiotics.
- Secondly, chemicals and nutrients are not the only substances that enter portal blood as it perfuses the intestines: it also accumulates products of the degradation of intestinal microorganisms such as inflammogenic lipopolysaccharide components of the bacterial cell wall (i.e. endotoxin). Since endotoxin delivery may increase during xenobiotic intoxication, immunological responses to co-absorbed endotoxin can exacerbate the hepato-toxicity of ingested chemicals.
- Thirdly, in addition to entry via the portal circulation, chemicals can access the liver via arterial blood that mixes with venous blood in the hepatic sinusoids. For example, inhaled tobacco constituents that enter via the lungs are efficiently delivered to the liver via the arterial route.
- Fourthly, the vast metabolic capacities of the liver also paradoxically heighten its vulnerability to chemical toxicity: by functioning as a miniaturised chemical factory that performs many diverse chemical modifications on foreign molecules, CYPs and other hepatic enzymes can inadvertently generate noxious metabolites that induce 'bioactivation-dependent' hepatotoxicity (Philip, and Burcham, 2014).

1.3.11. Liver

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, fight against disease, nutrient supply, energy provision and reproduction (Sharma et al., 1991). The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for the overall health and

well being (Subramaniam and Pushpangadan, 1999) This gland plays a major role in metabolism and has a number of functions in the body, including glycogen storage, decomposition of red blood cells, plasma protein synthesis, hormone production, and detoxification. It lies below the diaphragm in the abdominal-pelvic region of the abdomen. It produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver's highly specialized tissues regulate a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions.

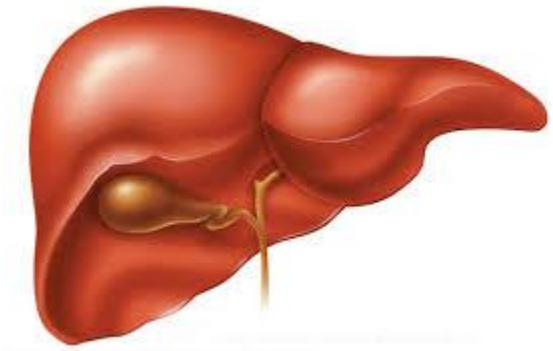


Figure 1.8*:* Liver

Anatomy:

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

Figure1.9: Anatomy of mice

1.3.11.1. Liver function tests

Liver function tests (**LFTs** or **LFs**) are groups of clinical biochemistry laboratory blood assays designed to give information about the state of a patient's liver.(Lee, Mary (2009-03-10)The parameters measured include prothrombin time (PT/INR), aPTT, albumin, bilirubin (direct and indirect), and others. Liver transaminases (AST or SGOT and ALT or SGPT) are useful biomarkers of liver injury in a patient with some degree of intact liver function. (Johnston, David (15 April 1999).Most liver diseases cause only mild symptoms initially, but these diseases must be detected early. Hepatic (liver) involvement in some diseases can be of crucial importance. This testing is performed by a medical technologist on a patient's serum or plasma sample obtained by phlebotomy. Some tests are associated with functionality (e.g., albumin), some with cellular integrity (e.g., transaminase), and some with conditions linked to the biliary tract (gamma-glutamyltransferase and alkaline phosphatase). Several biochemical tests are useful in the evaluation and management of patients with hepatic dysfunction. These tests can be used to detect the presence of liver disease, distinguish among different types of liver disorders, gauge the extent of known liver damage, and follow the response to treatment. Some or all of these measurements are also carried out (usually about twice a year for routine cases) on those individuals taking certain medications — anticonvulsants are a notable example — to ensure the medications are not damaging the mice’s liver.

Albumin:

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

Aspartate transaminase:

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

SGPT test:

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

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

SGPT levels may be higher than normal also if:

- drink too much alcohol.
- mononucleosis.
- chronic liver infection or inflammation.

- gallbladder inflammation, such as may caused by gallstones.
- a gallbladder infection.
- congested blood flow through the liver due to heart failure.

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 U/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 U/L, the differential can include alcohol toxicity, viral hepatitis, drug-induced level, liver cancer, sepsis, Wilson's disease, post-transplant rejection of liver, autoimmune hepatitis, and steatohepatitis (nonalcoholic). AST/ALT levels elevated minorly may be due to rhabdomyolysis, among many possibilities.

Alkaline phosphatase:

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

ALP - blood test

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

Normal Range:

The the range of activity for 306 apparently normal adult mice was **10–210 mU/ml** (international milliunits/ml), with a mean of 67 and a standard error of 1·7.

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

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

Higher-than-normal ALP levels

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

Lower-than-normal ALP levels

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

Other conditions for which the test may be done:

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

Chapter-2
Plant Introduction

Introduction to Plant

2.1 Plant information:

Stephania japonica (Thunb). (Syn S. harnendifolia) is a species under the genus of climbers belonging to family Menispermeceae. It is used in traditional medicinal practices and is locally known as Tubuki lota or Goldua having high medicinal value .*Stephania japonica* are used for treatment of fever, diarrhea, dyspepsia and urinary diseases.(M. Nishanthi et al,2011)

2.2 Description of *stephania japonica*

Scientific name: *Stephania japonica* (Thunb.) Miers.

Synonyms: *S. hernandifolia Walp., Menispermum japonicum Thunb.*

Family: Menispermaceae

Bengali/Vernacular Name: Akanadi, Nimuka, Maknadi.

Tribal Name: Tung Nah Way, Thaya Nuya (Marma).

English Name: Tape-vine.

Taxonimic Position

Division: Magnoliophyta

Class: Magnoliopsida

SubClass: eudicots

Order: Ranunculales

Family: Menispermaceae

SubFamily: Menispermoideae

Tribe: Tiliacoreae

Genus: Stephania

Species:*Stephania japonica* (Morse, 2016)

Description:

A dioecious vine without prickles. Greenish small flowers form on compound umbels, growing from the leaf axils in the warmer months. Inflorescences are 4 to 8 cm long. The fruit is an oval shaped, orange or red drupe, 2 to 5 mm long. A feature of this plant is the peltate leaves, (the stem is attached to the leaf, away from the leaf edge).

Leaf:

The leaf is distinctly dorsiventral and uniquely differentiated into adaxial and abaxial side. The adaxial and abaxial layers are thin with small squanish cells. The adaxial part of the lamina consists of a less prominent slightly thick part with a small vascular strand. This part represents the midrib.The total thickness of the leaf in the midrib region is 1.1mm; thickness of the abaxial midrib is 300Hm. The large part is 100hm thick.

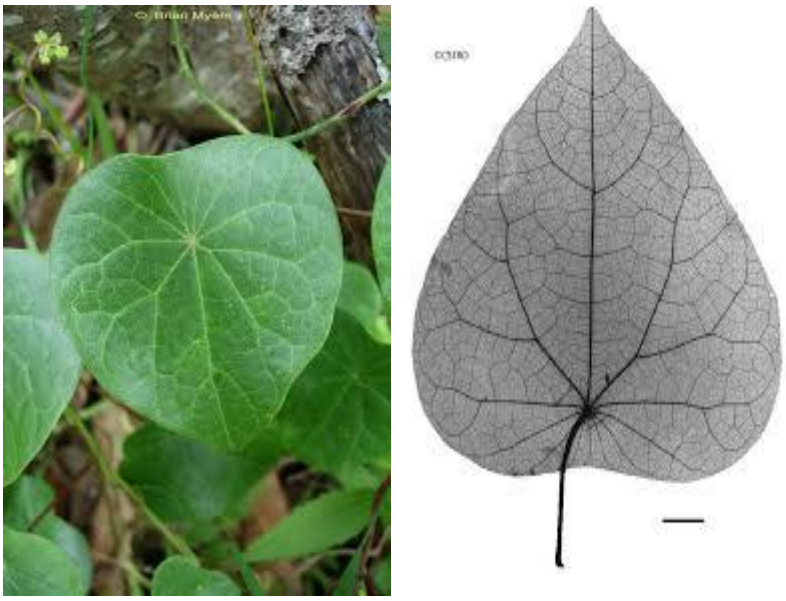


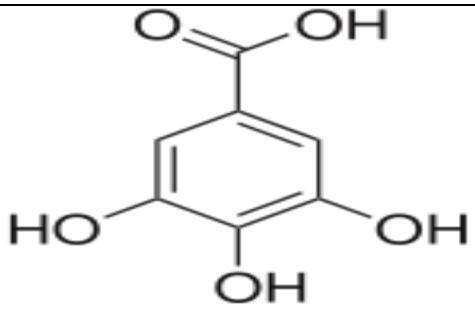
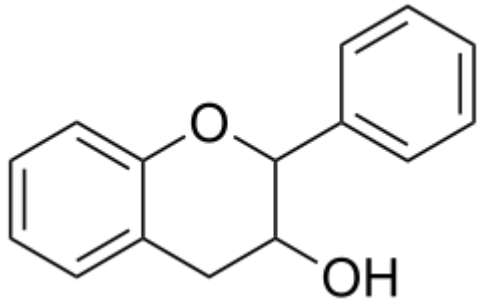
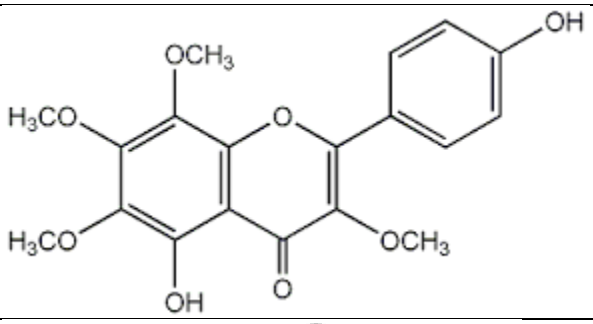
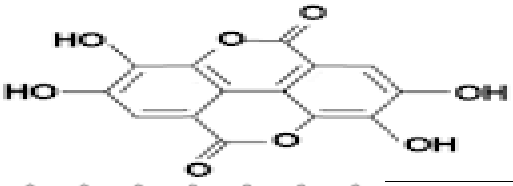
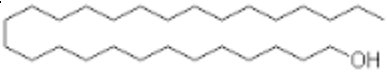
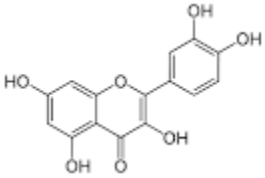
Figure 2.1: *Stephania japonica*

2.3 Geographical Distribution:

It is native to eastern and southern Asia and Australasia.In bangladesh,it is grown inmany areas(Chuadanga.Chittagong).(Rahman, 2011)

2.4 Chemical composition

Leaves contain tannin,alkaloid,and the flavanol, calycopterin, calycopterin-4-methyl ether, 3'oxymethylcalycopterin, 4'-O-methylcalycopterin, n-octacosanol, ellagic acid, quercetin and proanthocyanidin. Flowers contain calycopterin and quercetin. Heartwood also contains calycopterin and quercetin along with gossypol. The plant also contains traces of albuminoids and minerals (Ghani, 2003; Rastogi & Mehrotra, 1993).

Types	Compound	Structure
Tannin	**$C_7H_{13}O_5$**	 **Gallic acid**
Flavanol	**$C_{15}H_{14}O_2$**	
calycopterin	$C_{19}H_{18}O_8$	
Ellagic acid	$C_{14}H_6O_8$	
n-octacosanol	$C_{28}H_{58}O$	
quercetin	$C_{15}H_{10}O_7$	

2.5 Medicinal Uses:

Stephania japonica (Thunb.) of Menispermaceae family are known as *Patha*, in Ayurveda, which is used in the treatment of various diseases like stomach pain, fever, skin conditions, cardiac pains etc.(Hullatti,2011)

Stephania japonica are used for treatment of fever, diarrhea, dyspepsia and urinary diseases. The alkaloid akanidine shows significant anti spasmodic activity on uterine spasms.The leaves of the plant are employed in treatment of convulsions, skin diseases, cough, asthma like symptoms and kidney disorders.

In Japan and Taiwan, decoction of the plant is used as a drink to treat malaria. In Indonesia, the roots are used to provide relief in stomach aches convulsions.From the ethnomedical information and folk claims it is observed that the plant Stephania japonicavar. Timoriensis have medicinal properties related to urolithiasis and convulsant which have not been scientifically validated, and only some of the phytochemical studies have been carried out and reported for the presence of Alkaloids, Flavonoids, Tannin, Saponins with ability to produce stable foam and steroids.

(M. Nishanthi et al,2011)

Chapter-3
Literature Review

Review of literature:

Phytochemical studies:

Phytochemical screening: The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents.Phytochemical screening of the extract was performed using the following reagents and chemicals.Alkaloids with Dragendorff,s reagent.;flavonois with the use of Mg and HCL;tannins with ferric chloride and potassiumdichromate solutions and saponins with ability to produce stable foam and steroids with Libermann- Burchard reagent.Reducing sugars with Benedict's reagent.These were identified by charachteristc color changes using standard procedures.(Ghanni,2003)

In vivo analgesicc screening:

Acetic acid induced writhing test: The analgesic activity of teh samples was also studied using asetic acid-induced writhing test model in mice.Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered intraperitonially 15 min before injection of acetic acid.After an interval of 5 min, the mice were obsereved for specific contraction of body referred to as 'writhing' for the next 10 min.(ahmed et al,2004)

In vitro tests for antioxidant screening

Free radical scavenging activity measured by 1,1-diphenyl-2-picryl-hydrazyl(DPPH): The free Radical Scavenging activity of MeOH extract based on the Scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl(DPPH) free radical was determined by the method described by Braca et al.(2001).Plant extract (0.1 ml) was added to 3 ml of a 0.004% MeOH solution of DPPH.Absorbance at %17 nm was determined after 30 min and the percentage inhibition activity was calculated from [(A0-A1)/A0]×100,where A0 is the absorbance of the control and A1 is the absorbance of the extract/standard.IC50 value was calculated from the equation of line obtained by plotting a graph of concentrations (µg mL^{-1}) Versus % inhibition.

(Rahman, 2011)

Evaluation of Post-Coital Pregnancy Interceptive Ac- tivity: Female rats exhibiting normal estrus cycle were se- lected for the study. These female rats were caged overnight with coeval males of proven fertility in the ratio of 2:1 and the vaginal smears of female rats were checked on the following morning. The day of presence of spermatozoa in the vaginal smear was considered as day 1 of pregnancy. Mated rats were isolated, randomized into various treatment groups and treated orally with the test agent or distilled water (vehi- cle) during first 7d of post-coitum. The animals were laparo- tomised under light ether anesthesia and sterile conditions on day 10 of pregnancy. Both horns of the uterus were observed for the number and status of implants and corpora lutea. The rats were allowed to recover and deliver after full term.25)

Determining Variations in Estrus Cycle: The estrus cycle of rats were monitored for 12d in two groups, viz., con- trol and dose of test substance with best activity. Acyclic rats and rats with prolonged cycles were screened and eliminated. The process included examination of vaginal smear from each animal under microscope to observe different phases and duration of the estrus cycle.2

(Mukherjee .et.al, 2006)

Chapter-4

Materials and Methods

4.1.1. Preparation of plant extraction

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

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

1. The residue portion over the filter

2. The filtered part

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

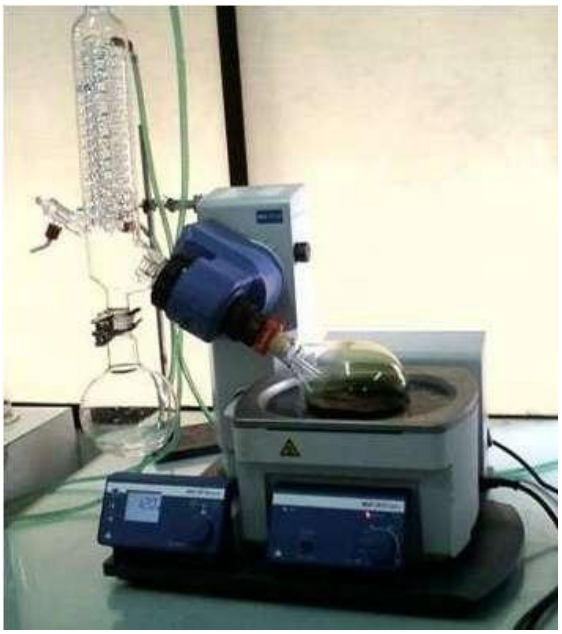


Figure 4.1. Rotary evaporator

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.

4.2. Experimental Animals

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

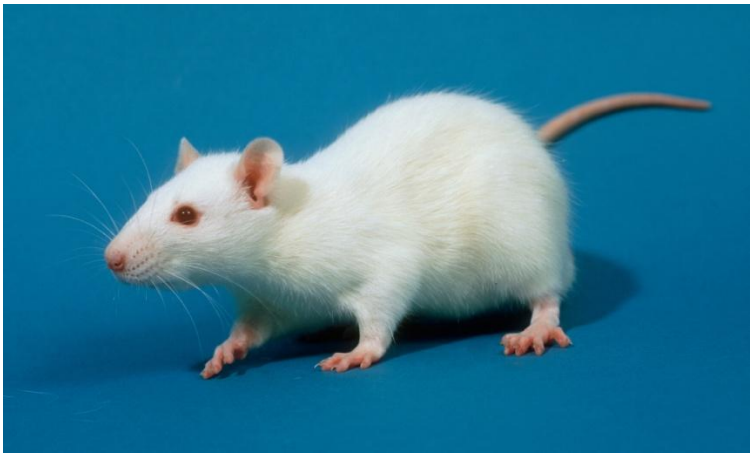


Figure 4.2.*Swiss albino* Mice

4.3. Equipments

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

4.4. Drugs and Chemicals

4.4.1. Chemical Agents

1. 5% CMC (Vehicle) 10ml/kg as negative control,

2. 0.3 mL of charcoal meal of distilled water suspension containing 10% gum acacia, 10% activated charcoal and 20% starch.

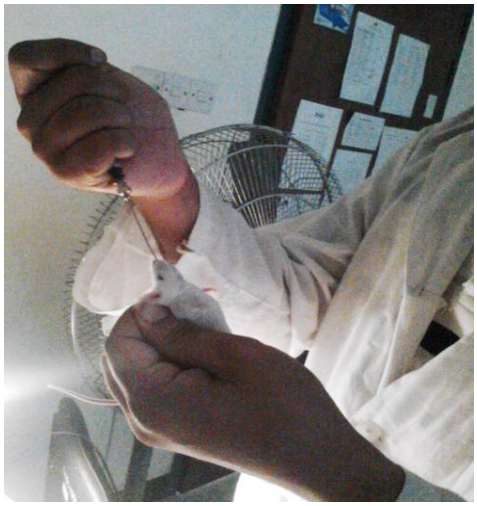


Figure 4.3. Oral administration into mouse.

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

4.5.4.2. Hole Board Test:

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

4.5.5. 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 *Stephania japonica* at 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 4.4 Open Field Test

4.5.5.2. Hole Board Test:

The hole board represents a combination of a hole board, originally designed to investigate explorative motivation in rodents (Perez G.R.M., et al., 1998) and later on modified to evaluate cognitive functions (Ohl and Fuchs, 1999; Ohl et al., 1998) The hole board itself consisted of a total of 16 holes, each 3 cm in diameter, were presented to the mouse in a flat space of 25 square centimeters. This experiment was carried out by the following method of Boisser and Simon, (1964). The animals were divided into negative control and test groups containing six mice in each group. Negative control group received vehicle (5% CMC solution) at a dose of 10 mg/kg body weight orally. The test groups received extracts *Stephania japonica* at the doses of 200,400 mg/kg & 800mg/kg body weight orally. Each of the animals was transferred carefully to one corner of the field and the number of ambulation (expressed as the number of holes passed), head dipping and numbers of head poking was recorded for a period of 5 minutes and post 30 minutes intervals and were compared with the control animals

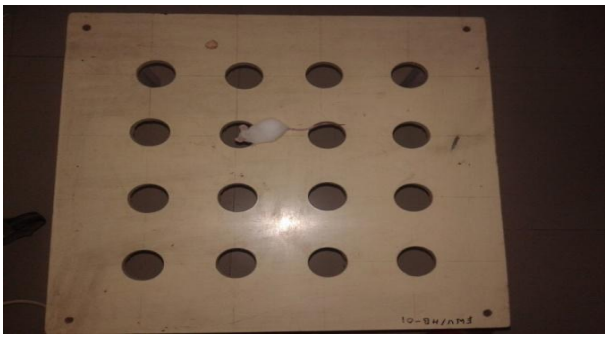


Figure 4.5. Hole Board Test

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%)
- Heparin
- Choloform

4.6.3 Doses Used for Toxicological Activity of the Extract:

4.6.3.1. Acute Toxicity Test:

Methanolic extracts of *Stephania japonica* at a dose of 1300mg/kg, 1600mg/kg and 32000mg/kg and 6400mg/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 *Stephania japonica* at 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 1300,1600 and 3200,6400mg/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 10 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 XS-800i** Hematology Analyzer ,National cancer institute , 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 (National cancer institute, Dhaka, Bangladesh)

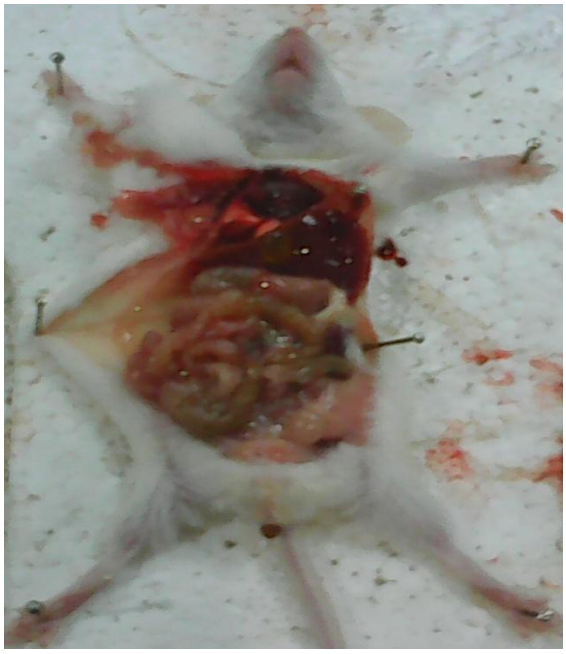


Figure 4.6. Mice Organ

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(National cancer institute, Dhaka, Bangladesh) 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.

Chapter 5
Result and Discussion

5. Result and Discussion

5.1 Formation of Tumor:

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

After 33 days a male mice which was administered with 200mg/kg methanolic extracts of *Stephania japonica* ,was appeared a large tumour upper the neck side.

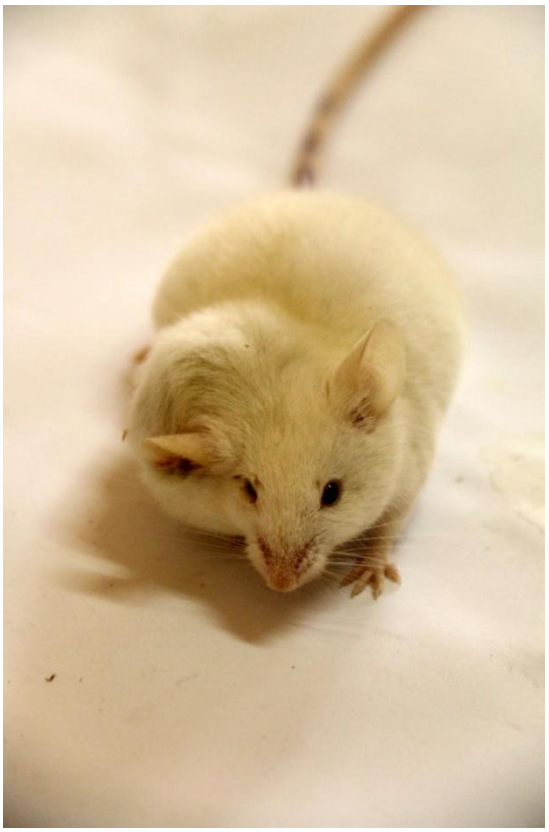


Figure5.1:SJ m2(04)

Later some of the mice appeared to have small lump on the body.The average size of the lump was 1cm.These mice were disected and organs (Heart.kidney,Liver,Lung,Pancreas) were kept in 10% Formalin.These organs are subjected to Histopathology test.They are suspected to have carcinogenic effect due to administration of the plant.It was observed that these tumor was formed among those mice who received 200mg/kg and 800mg/kg methanolic extracts of *Stephania japonica.*

Figure 5.2:SJ M 2(10)

Figue 5.3: SJ M8(7)

***Stephania japonica*:**

Crude extract of ***Stephania japonica*** 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.

5.2. CNS Activity Test of Methanolic Extract of *Stephania japonica*

5.2.1. Open Field Test:

CNS of the methanolic extract of the bark part of the plant *Stephania Japonica* studied in different doses (200, 400 and 800mg/Kg body weight) of the crude extract, using diazepam as a positive control. The extract produced effects at doses of 200, 400 and 800 mg/kg body weight respectively (Table5.2, 5.3 and 5.4 and Fig. 5.2, 5.3 and 5.4). The result was found to statistically significant. The experimental findings that are noted are below-

Table 5.1: CNS Activity of plant extract of *Stephania japonica* 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	96.33±5.25	102.5±3.46	104.4±2.31	101.9±2.34	101.6±2.01
Crude extract of *Stephania japonica*	200mg/kg	49.3±4.14	42.33±3.09	36±3.75	31.83±3.36	25±3.73
Crude extract of *Stephania japonica*	400mg/kg	39±1.94	34.33±1.52	29.33±1.87	24.66±1.76	20.6±1.56
Crude extract of *Stephania japonica*	800mg/kg	48.14±1.24	42.17±1.2	37.14±1.54	32.28±1.42	28.28±1.17
Positive control, Diazepam	1mg/kg	121.83±1.1	69.33±1.12	53±1.81	35.67±1.17	27.83±1.7

Each value is the mean ± SEM for 10 mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

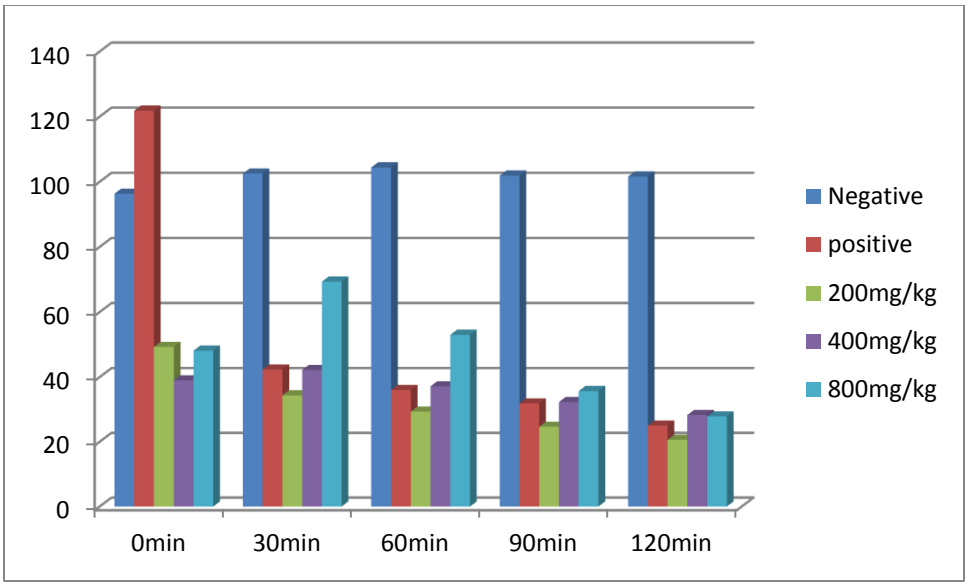


Figure 5.4: Graphical Presentation of CNS Activity of plant extract of *Stephania japonica* by Open Field Test (Peripheral Locomotion) in Mice.

Table 5.2: CNS Activity of plant extract of *Stephania japonica* by Open Field Test (Central Locomotion) in Mice.

Groups	Dose	No. of Central Locomotion				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	10.7± .83	9.33±.93	11.3± 0.97	10.4 ±0.87	11.0±0.94
Crude extract of *Stephania japonica*	200mg/kg	4.5±0.43***	3.67±0.42*	2.67±0.21*	2.3±0.21*	2.16±0.16*
Crude extract of *Stephania japonica*	400mg/kg	3.66±0.33***	3.33±0.21*	2.67±0.21*	2.33±0.21**	2.16±0.16*
Crude extract of *Stephania japonica*	800mg/kg	5.4±0.53	4.42±0.37*	3.7±0.42*	2.7±0.19*	2.14±0.14*
Positive control, Diazepam	1mg/kg	20.67±1.05	9.5±0.76	6.17±0.6	4.17±0.6	3.33±0.42*

Each value is the mean ± SEM for 10 mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

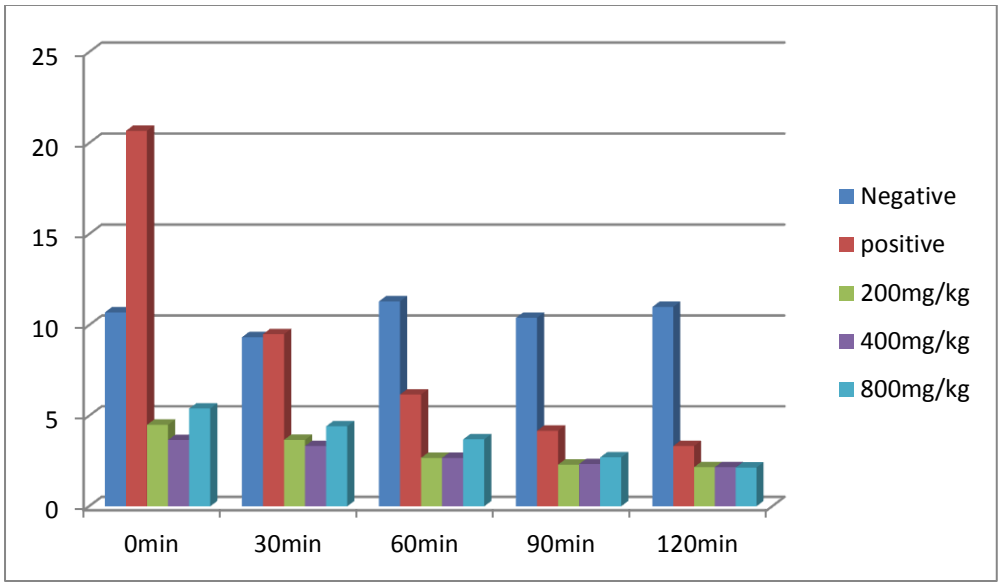


Figure 5.5: Graphical Presentation of CNS Activity of plant extract of *Stephania japonica* by Open Field Test (Central Locomotion) in Mice.

Table 5.3: CNS Activity of plant extract of *Stephania japonica* 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	10.70±0.96	13.10±1.03	9.4±0.87	7.1±0.89	8.9±0.78*
Crude extract of *Stephania japonica*	200mg/kg	4.5±0.42	4±.44*	3±.52	2±.58*	0.83±.4*
Crude extract of *Stephania japonica*	400mg/kg	7±.81	5.4±.57	3.7±.28*	1.5±.42*	0.42±.29*
Crude extract of *Stephania japonica*	800mg/kg	3.8±.8	3.4±.51	4.2±0.49*	3.4±.5*	6.2±.80
Positivecontrol, Diazepam	1mg/kg	22.17±1.08	8.83±0.31*	6.17±0.31*	4.33±0.33*	3.17±0.48*

Each value is the mean ± SEM for 10 mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

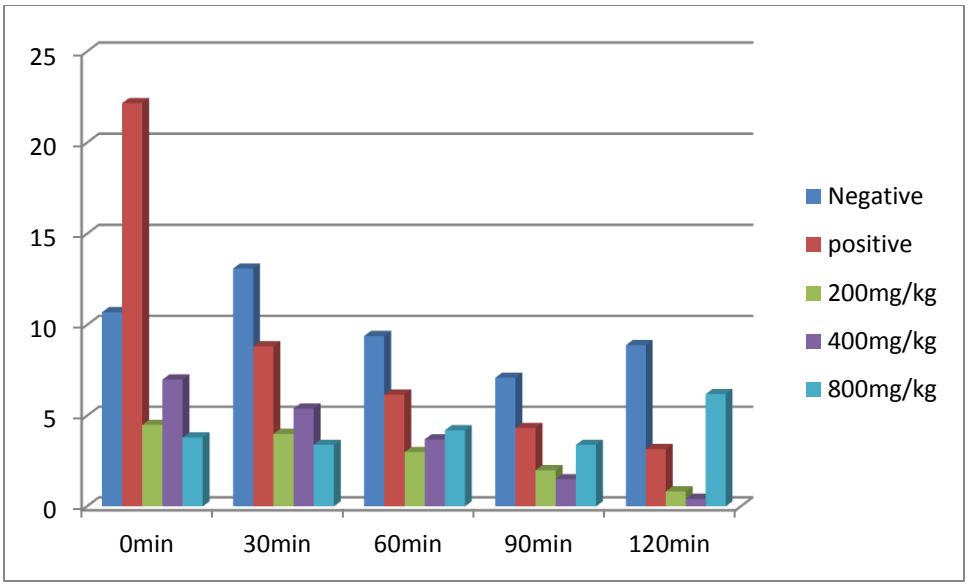


Figure 5.6: Graphical Presentation of CNS Activity of plant extract of *Stephania japonica* by Open Field Test (Leaning) in Mice.

5.2.2Hole Board Test:

CNS of the methanolic extract of the bark part of the plant *Stephania japonica* studied in different doses (200, 400 and 800mg/Kg body weight) of the crude extract, using diazepam as a positive control. The extract produced effects at doses of 200, 400 and 800 mg/kg body weight respectively (Table5.5, and Fig. 5.5). The result was found to statistically significant. The experimental findings that are noted are below-

Table 5.4: CNS Activity of plant extract of *Stephania japonica* by Hole Board Test in Mice.

Hole Board Test

Groups	Treatment	Dose	No. of head dipping	No.of head poking
Negative control	5% CMC	10ml/kg	49.3±1.29	59.8±2.78
Group-1	Crude extract of *Stephania japonica*	200mg/kg	47±1.39	56.8±2.54
Group-2	Crude extract of *Stephania japonica*	400mg/kg	42.6±1.44	52.5±2.57
Group-3	Crude extract of *Stephania japonica*	800mg/kg	38.8±1.64	46.2±2.59
Positive control	Diazepam	1mg/kg	29.83±1.01	15.67±0.67

Each value is the mean ± SEM for 10 mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

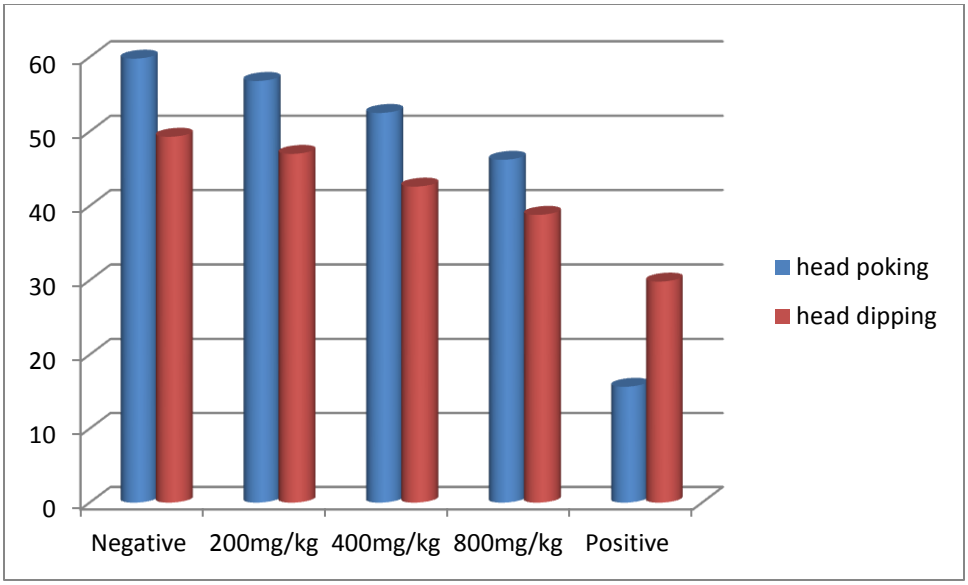


Figure 5.7: Graphical Presentation of CNS Activity of plant extract of *Stephania japonica* by Hole Board Test in Mice.

5.3. Acute and Chronic Toxicity Test:

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

5.3.2. Chronic Toxicity Test:

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

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

In the chronic study of methanolic extract of *Stephania japonica* 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 *Stephania japonica* 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 *Stephania japonica*.

Table 5.5 : Effect of methanolic extract of *Stephania japonica* on body weight in mice

Treatment group	Initial body wt	Final body wt.	No.of death
Negative Control	17.5±0.6	30.19±1.93	0
***Stephania japonica* 200mg/kg**	31.9±0.95	45.2±1.124	5
***Stephania japonica* 400mg/kg**	35.1±1.03	47.6±0.51	5
***Stephania japonica* 800mg/kg**	30.9±0.86	45.2±0.97	5

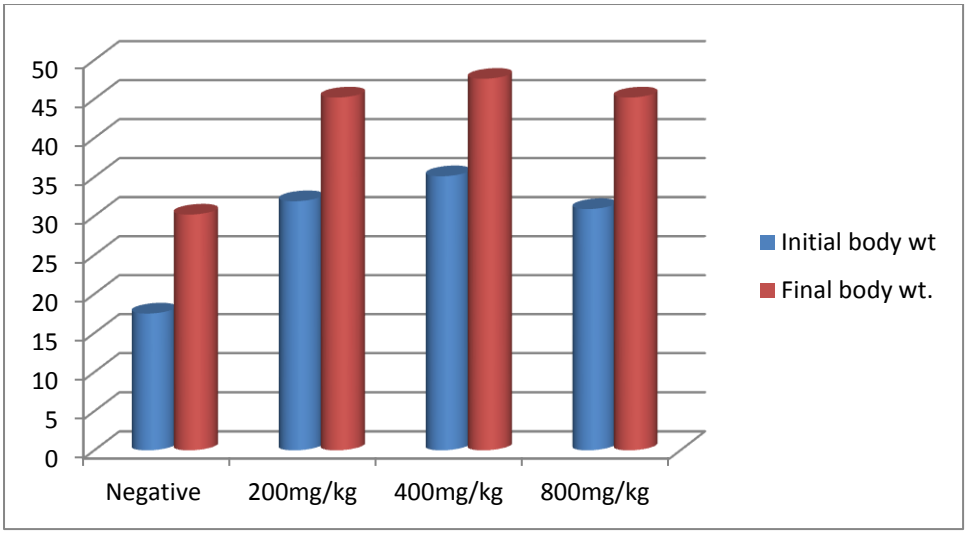


Figure 5.8: Graphical Presentation of Effect of methanolic extract of *Stephania japonica* on body weight in mice

Table 5.6: Effect of *Stephania japonica* on the count of WBC (White Blood Cell)

Treatment group	TotalWBC $10^3/mm^3$(n)	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Negative control group(Male)	5.43	18.32	76.18	4.15	0.67	0.68
Stephania japonica(200mg/kg)	9.99	5.33	28.82	2.53	0.5	0.5
Stephania japonica(400mg/kg)	5.87	8.62	9.02	4.22	0.64	0.34
Stephania japonica(800mg/kg)	6.6	13.04	63.12	2.74	0.2	1.3

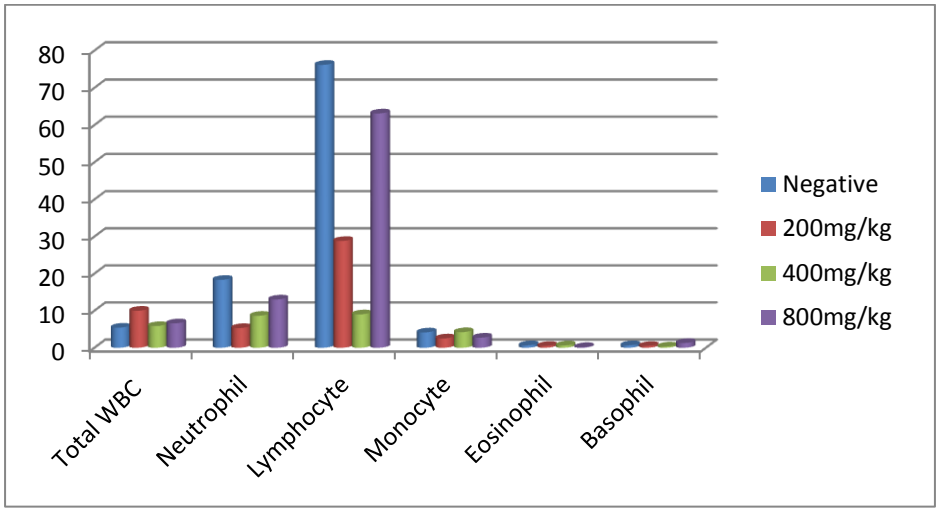


Figure 5.9 : Effect of *Stephania japonica* on the Different count of WBC (White Blood Cell)

Table 5.7 : Effect of *Stephania japonica* on the count of RBC (Red Blood Cell)

Treatment group	Total RBC$10^6/mm^3$(n)	Haemoglobin	HCT	MCV	MCH	MCHC	RDW
Negative control(Male)	8.64	14.45	42.95	49.88	16.75	33.73	24.45
Stephania japonica(200mg/kg)	8.06	12.12	41.7	51.08	14.85	29	23.45
Stephania japonica(400mg/kg)	7.75	12.84	40.98	50.8	16.72	32.52	23.26
Stephania japonica(800mg/kg)	8.05	12.66	43.14	53.8	15.74	29.38	21.46

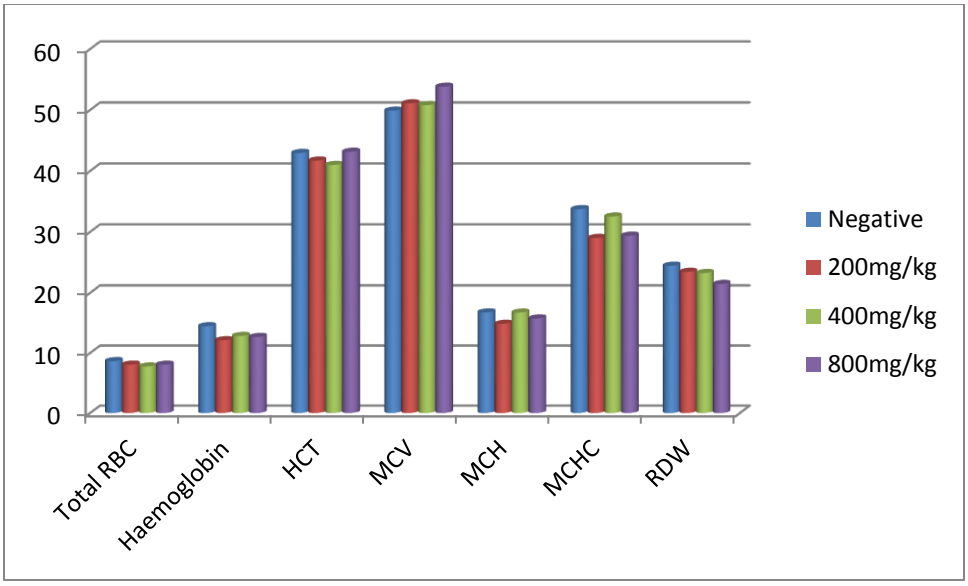


Figure 5.10: Effect of *Stephania japonica* on the count of RBC (Red Blood Cell)

Table 5.8: Effect of *Stephania japonica* on Platelet count on the CBC (Count Blood Cell) Test

Treatment Group	Platelet $10^3/mm^3$(n)
Negative Control group	949.33
***Stephania japonica* (200mg/kg)**	1131
***Stephania japonica* (400mg/kg)**	901.8
***Stephania japonica* (800mg/kg)**	790.6

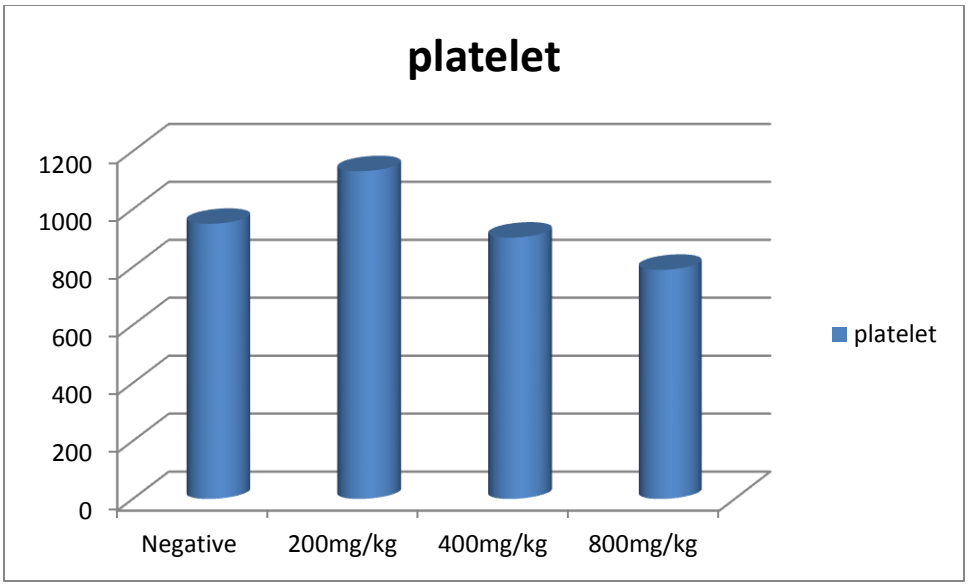


Figure 5.11: Effect of *Stephania japonica* on Platelet on the CBC (Count Blood Cell) Test

Table 5.9: Effect of *Stephania japonica* on the Liver Function Test

Treatment group	SGPT (U/L)	SGOT (U/L)	SALP (U/L)
Negative control group(Male)	31	23.55	169.3
Stephania japonica(200mg)	177.17	135.67	180
Stephania japonica(400mg)	48.6	15.4	154.4
Stephania japonica(800mg)	113.8	69.2	137

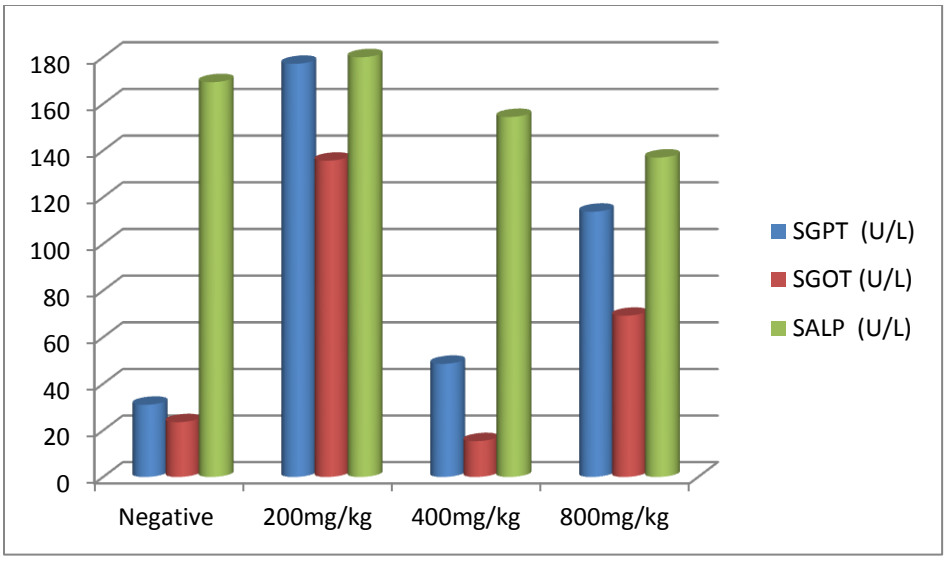


Figure 5.12: Effect of *Stephania japonica* on the Liver Function Test

Chapter 6
Conclusion

6. Conclusion

Traditional medicines are mostly utilized by means of the natural products isolated from natural resources such as plant extracts. Pharmacological studies always reveal the potential medicinal properties of plants of our surroundings.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.

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

The aim of the study was also to investigate the possible toxicity of the plant *Stephania japonica* 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 result shows that the toxic effect of methanolic extract of *Stephania japonica* is danger in mice that is significant change with dose when compare with negative control From the present investigation, it can be concluded that the methanolic extract of *Stephania japonica* exhibited Depressant activity,and shows toxicity effects in acute and chronic toxicity studies in mice.

Chapter 7
Reference

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Annexure

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

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