Study of Pharmacological and Toxicological Activities of *Trapa bispinosa*

Submitted By

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A Thesis Report Submitted to the Department of Pharmacy, East West University, In Partial Fulfillment of the Requirements for the Degree of Masters of Molecular & Clinical Pharmacy

Declaration by the Candidate

I, **Nusrat Jahan**, hereby declare that, **'Study of Pharmacological and Toxicological Activities of *Trapa bispinosa*'** submitted by me to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the Degree of Masters of Pharmacy (M.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 and Toxicological Activities of *Trapa bispinosa*'**, submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the Degree of Masters of Pharmacy , was carried out by Nusrat Jahan , ID No. 2014-1-79-009 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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Dr. Shamsun Nahar khan

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Endorsement by the Chairperson

This is to certify that the entitled **'Study of Pharmacological and Toxicological Activities of *Trapa bispinosa*'** , is a genuine research work carried out by Nusrat Jahan, ID No. 2014-1-79-009under 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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At first, I would like to express my gratitude to Almighty God for giving me the strength and opportunity to complete my dissertation within the schedule time successfully.

I feel proud to express my deep sense of gratitude to my reverend teacher, guide and supervisor Dr.Shamsun Nahar Khan, Associate professor and chairperson**,** Department of Pharmacy, East West University, Dhaka, Bangladesh, for her day to day supervision, dexterous management, adept analysis, keen interest, optimistic counseling and unremitting backup.

I would like to convey deepest love and obedience to my parents for their support, inspiration and guiding me all through my life until today, which keeps me strong and firm to do the things I needed to do.

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I am thankful to the laboratory instructors for their kind support during the laboratory work.

Nusrat Jahan

ABSTRACT

Purpose: The research work was carried out to determine the Study of Pharmacological and Toxicological Activities of *Trapa bispinosa.*

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

Result: The 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 *Trapa bispinosa* (200mg/kg, 400mg/kg & 600mg/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 when it is compared to the standard drug.

The aim of the study was also to investigate the possible toxicity of the plant *Trapa bispinosa* 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 *Trapa bispinosa* may have several pharmacological activities but to prove the hypothesis it need further higher studies.

Keywords: *Trapa bispinosa*, Neuropharmacological effect and Toxicity test.

DEDICATION

This research paper dedicated

To

My beloved parents

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Chapter 1
Introduction

1.1.Medicinal Plant

Throughout history plants have been used by human beings for medicinal purposes and even in modern times have formed the basis of many pharmaceuticals in use (Schmidt et. al.,2008). 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. A recent review has shown that approximately 25% of modern medications have been plant derived, while 75% of new drugs against infectious diseases that have arrived between 1981 and 2002 originated from natural sources (Bedoya et.al.,2009) 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). 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 plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes (Ghani, 2003).

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

1.1.3 Medicinal plants & Traditional Medicine Practice in Bangladesh

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

Traditional medical practice among the tribal people is mainly based on the use of plant and animal parts and their various products as items of medicine. The medicaments, prepared from plant materials and other natural products sometimes also include some objectionable substances of animal origin. They are dispensed in a number of dosage forms like infusions, decoctions, pastes, moulded lumps, powders, dried pills, creams and poultices. Diets are strictly regulated .Since indigenous peoples have a long history and expertise in the use of medicinal plants, it is impotant that their plant usage be documented as the basis for the development of lead compounds before this knowledge is lost due to the influences of modern civilization. Bangladesh has a number of indigenous people or tribes including the Chakmas, Garos, Santals, Marmas, Tripuras and others (Hussain et.al., 2012).

1.1.4. Nervous System

The human nervous system is perhaps the most complex system of any organism. The nervous system consists of the brain, spinal cord, sensory organs, and all of the nerves that connect these organs with the rest of the body. Together, these organs are responsible for the control of the body and communication among its parts. 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 possible routes. The nervous system has two major divisions: the central nervous system (CNS) and the peripheral nervous system (PNS). The brain and spinal cord form the control center known as the central nervous system (CNS), where information is evaluated and decisions made. The sensory nerves and sense organs of the peripheral nervous system (PNS) monitor conditions inside and outside of the body and send this information to the CNS. Efferent nerves in the PNS carry signals from the control center to the muscles, glands, and organs to regulate their functions. Efferent nerves in the PNS carry signals from the control center to the muscles, glands, and organs to regulate their functions.

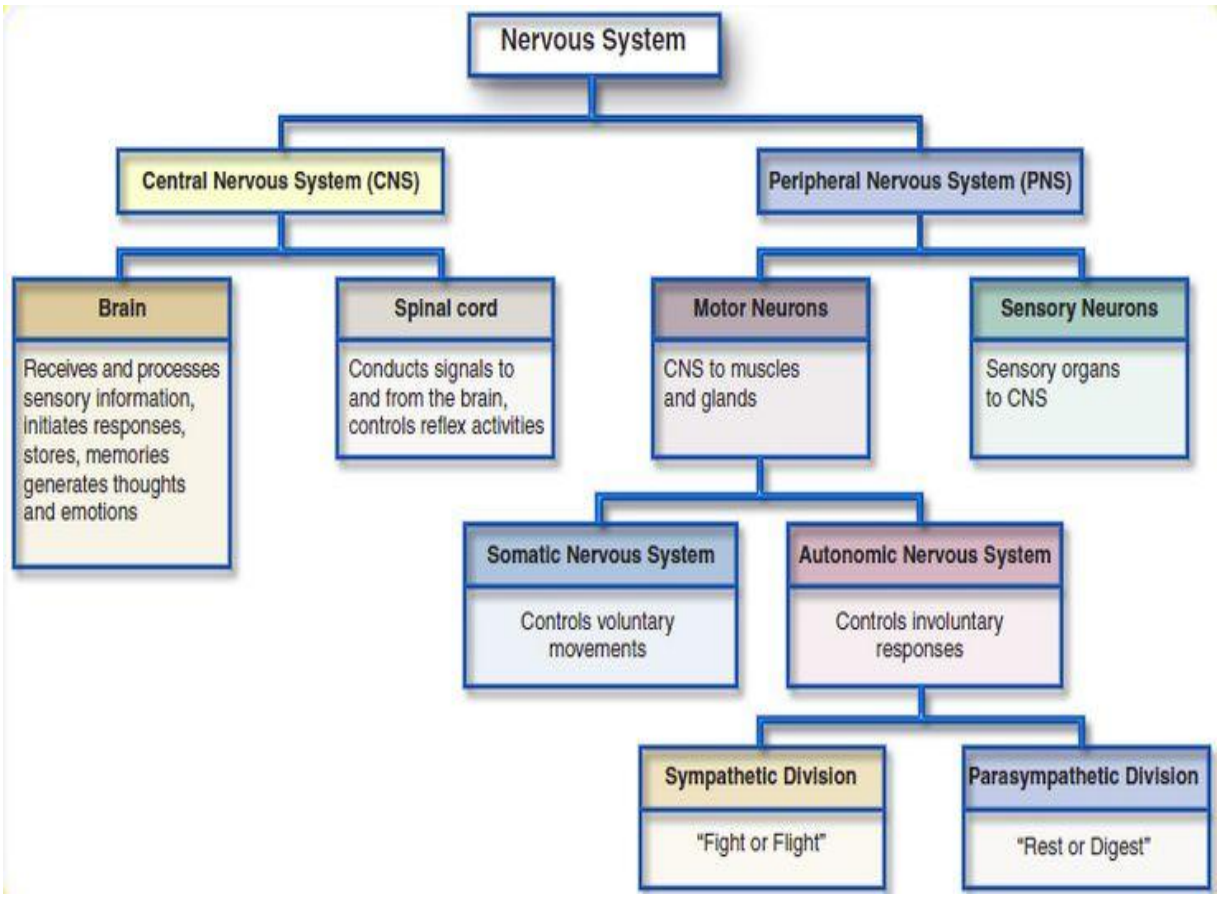


Figure-1: Organization of the Human Nervous System.

1.1.4.1. 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).

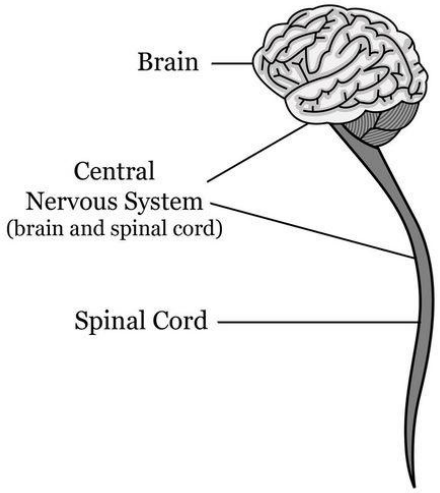


Figure-2: Central Nervous System

Parts of Central Nervous System

- Brain
- Medulla
- Pons
- Cerebrum
- Cerebellum
- Spinal cord

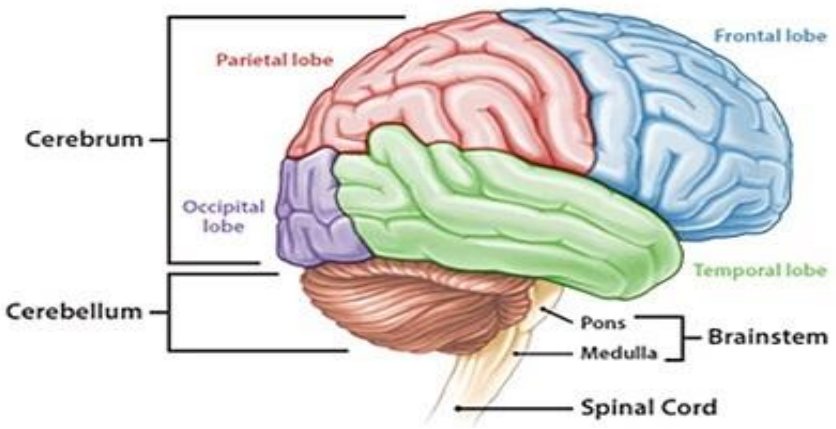


Figure-3: Human Brain

1.1.4.2. 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.

1.1.4.3. Nerve cells

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

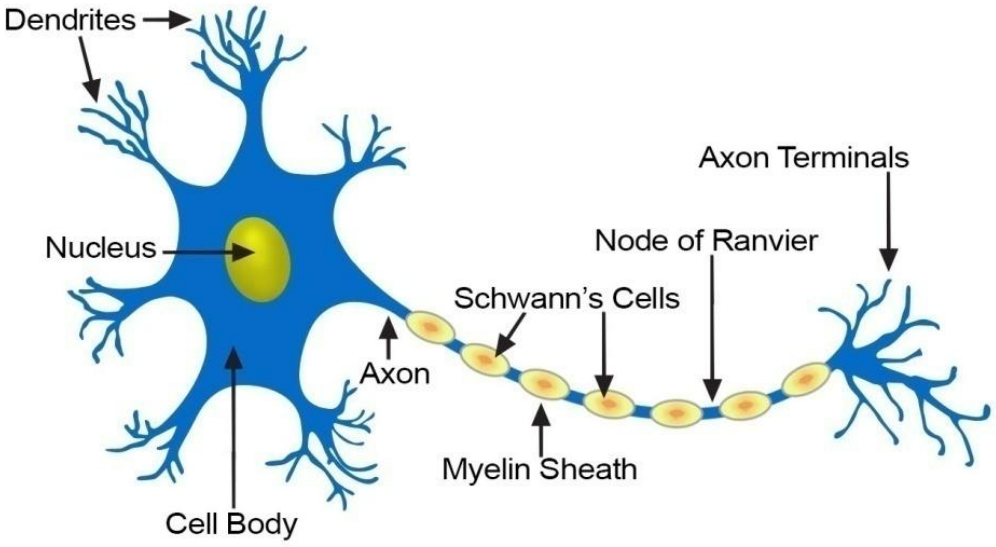


Figure-4: Neuron

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 unmylinated regions between the myelin segments are called the nodes of ranvier. Thus, axons are of two types, myelinated and non-myelinated (Martini et.al., 2003).

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).

1.1.4.4. 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.

1.1.4.5. 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).

1.2. Toxicity aspects of use of herbal preparations

Currently, there is an ongoing world-wide "green" revolution which is mainly premised on the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs (Williamson et al,. 1996). Many writers claim that it is assumed that "all things natural are good" (Gaillard and Pepin, 1999) and, generally, the extensive traditional use of herbal products is not assumed to be based on a comprehensive well documented logic, but rather on empirical wisdom accumulated over many years, often arrived at through trial and error and transmitted orally from generation to generation. This traditional methodology has enabled those herbal medicines producing acute and obvious signs of toxicity to be well recognized and their use avoided. However, the premise that "traditional use of a plant for perhaps many hundreds of years establishes its safety does not necessarily hold true". The more subtle

and chronic forms of toxicity, such as carcinogenicity, mutagenicity, and hepatotoxicity, may well have been overlooked by previous generations and it is these types of toxicity that are of most concern when assessing the safety of herbal remedies (Williamson et.al., 1996).

1.2.1. Causes of toxicity with herbal products

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

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

• In the first category, as previously mentioned, the toxicity may be as a result of exposure to intrinsic ingredients of some medicinal plants. Examples of some more important classes of ingredients implicated here include: pyrrolizidine alkaloids, which are said to be hepatocarcinogens; aristolochic acid I, said to be mutagenic and carcinogenic; phorbol esters, which are tumor promoters and vesicant to the skin; carboxy actractyloside, a deadly toxic compound; amygdalin, a cyanogenic compound with many undesired effects; etc.. In addition, several studies conducted on flavonoids indicate that, besides their apparently beneficial health effects, they may also induce mutagenicity and genotoxicity (e.g. quercetin) in both bacterial and mammalian experimental systems (Gaillard and Pepin,1999; Tomlinson and Akerele, 1998).

• The second category of causes of toxicity of herbal medicines is more extrinsic or non-associated with the plant active constituents. In this category, the toxicity is a result of exposure to plant products contaminated with excessive or banned pesticides, microbial contaminants, heavy metals or chemical toxins, or with substituted ingredients. The pesticide, heavy metal and microbial contaminants may be linked to the source, collection or processing of the herbal materials (e.g.in contaminated environments). (Skibola and Smith, 2000).

1.2.2. Toxicology

Toxicology is a branch of biology, chemistry, and medicine concerned with the study of the adverse effects of chemicals on living organisms. It also studies the harmful effects of chemical, biological and physical agents in biological systems that establish the extent of damage in living organisms. The relationship between dose and its effects on the exposed organism is of high significance in toxicology.

1.2.3. Toxicity

Toxicity is the degree to which a substance can damage an organism. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ such as the liver (hepatotoxicity). By extension, the

word may be metaphorically used to describe toxic effects on larger and more complex groups, such as the family unit or society at large.

A central concept of toxicology is that effects are dose-dependent; even water can lead to water intoxication when taken in too high a dose, whereas for even a very toxic substance such as snake venom there is a dose below which there is no detectable toxic effect. Toxicity is species-specific, making cross-species analysis problematic.

1.2.4. Exposure

In order for a chemical to produce a biological effect, it must first reach a target individual. Then the chemical must reach a target site within the body (toxicokinetics). Toxicity is a function of the effective dose of a foreign chemical at its target site, integrated over time. Individual factors such as body weight will influence the dose at the target site.

1.2.5. Route of Exposure

The route (site) of exposure is an important determinant of the ultimate dose. The route of exposure may be important if there are tissue-specific toxic responses. Toxic effects may be local or systemic Different routes may result in different rates of absorption like

- ✓ Dermal (skin)
- ✓ Inhalation (lung)
- ✓ Oral ingestion (Gastrointestinal)
- ✓ Injection (Parenteral)

1.2.6. 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).

1.2.7. 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).

1.2.8. Evaluation of herbal toxicity

Herbal toxicity can be evaluated by

(1) observing human or animal populations exposed to the plant material,

(2) administering the plant medicine to animals under controlled conditions and observing the effects (in vivo) and

(3) exposing cells, sub-cellularfractions or single-celled organisms to the plant material (in vitro)(Timbrell, 2002).

1.3. Hematology

In hematology we deal with the essentials of blood and the tissues for the forming blood. 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 (Ramsay, 1999).

1.3.1. 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 (Hajdu, 1998).

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).

1.3.2. Plasma

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

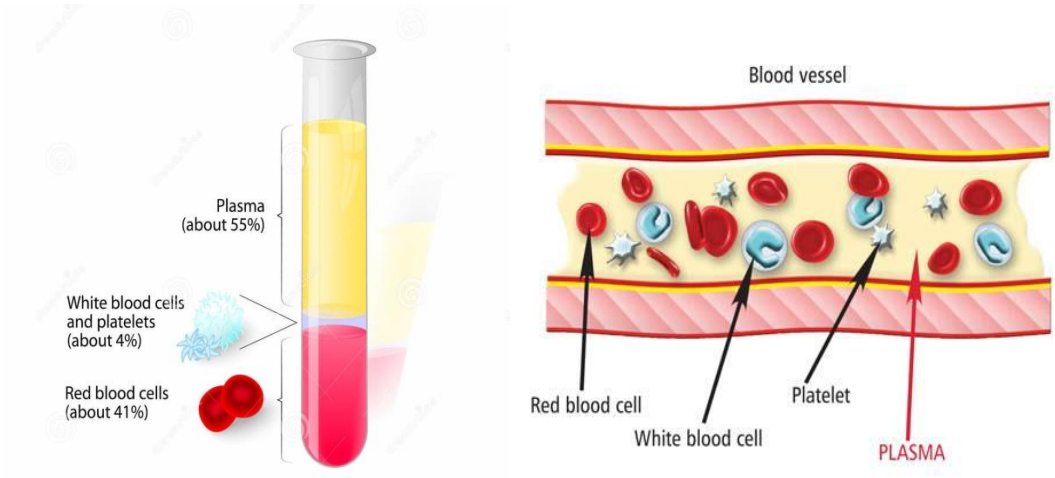


Figure-5: Plasma of the Blood

1.3.3. Cellular Elements

1.3.3.1. Red Blood Cell

RBCs have a shape of a disk that appears to be "caved in" or almost flattened in the middle; this is called bi-concave. This bi-concave shape allows the RBC to carry oxygen and pass through even the smallest capillaries in the lungs. This shape also allows RBCs to stack like dinner plates and bend as they flow smoothly through the narrow blood vessels in the body. RBCs lack a nucleus (no DNA) and no organelles, meaning that these cells cannot divide or replicate themselves like the cells in our skin and muscles. RBCs have a short life span of about 120 days, however, as long as our myeloid tissue is working correctly, we will produce about 2-3 million RBCs per second. That is about 200 billion a day! This allows us to have more to replace the ones we lose. The main component of the RBC is hemoglobin protein, of which there are about 250 million per cell. The word hemoglobin comes from "hemo" meaning blood and "globin" meaning protein. Hemoglobin is composed of four protein subunits: polypeptide globin chains that contain anywhere from 141 to 146 amino acids. Hemoglobin is responsible for the cell's ability to transport oxygen and carbon dioxide. Normal range of RBC (8-16)× $10^6 mm^3$ (Robert et.al., 2006).

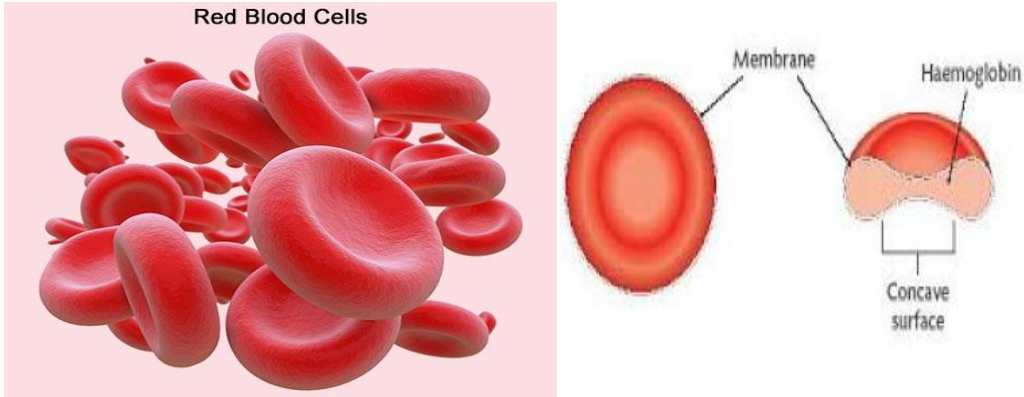


Figure-6: Red Blood Cell

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.

The hemoglobin test is a commonly ordered blood test and is almost always done as part of a complete blood count (CBC). Common reasons or conditions for ordering the hemoglobin test include:

- Symptoms such as fatigue, feelings of poor health, or unexplained weight loss
- Signs of bleeding are present
- Before and after major surgery
- During pregnancy
- Presence of chronic kidney disease or many other chronic medical problems
- Monitoring of anemia and its cause
- Monitoring during treatment for cancer
- Monitoring medicines that may cause anemia or low blood counts

Normal results for adults vary, but in general are:

- Male: 13.8 to 17.2 grams per deciliter (g/dL)
- Female: 12.1 to 15.1 g/dL

Lower than Normal Hemoglobin

Low hemoglobin level may be due to:

- Anemia due to red blood cells being destroyed earlier than normal (hemolytic anemia)
- Anemia (various types)
- Bleeding from digestive tract or bladder, heavy menstrual periods
- Chronic kidney disease
- Bone marrow being unable to produce new blood cells. This may be due to leukemia, other cancers, drug toxicity, radiation therapy, infection, or bone marrow disorders
- Poor nutrition

- Low level of iron, folate, vitamin B12, or vitamin B6
- Other chronic illness, such as rheumatoid arthritis

Higher than Normal Hemoglobin

High hemoglobin level is most often due to low oxygen levels in the blood (hypoxia), present over a long period of time. Common reasons include:

- Certain birth defects of the heart, present at birth (congenital heart disease)
- Failure of the right side of the heart (cor pulmonale)
- Severe COPD
- Scarring or thickening of the lungs (pulmonary fibrosis) and other severe lung disorders
- A rare bone marrow disease that leads to an abnormal increase in the number of blood cells (polycythemia vera)
- The body not having as much water and fluids as it should (dehydration)

Hematocrit (HCT)

The hematocrit (Ht or HCT, British English spelling haematocrit), also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood cells in blood. It is normally 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. Anemia refers to an abnormally low hematocrit, as opposed to polycythemia, which refers to an abnormally high hematocrit. Both are potentially life-threatening disorders (Purves, 2004).

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. The normal reference range is typically 80-100 femtolitres.

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 31picograms/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.

1.3.3.2. White Blood Cell

White blood cells are different from red cells in the fact that they are usually larger in size 10-14 micrometers in diameter. White blood cells do not contain hemoglobin which in turn makes them translucent. Many times in diagrams or pictures white blood cells are represented in a blue color, mainly because blue is the color of the stain used to see the cells. White blood cells also have nucleii, that are some what segmented and are surrounded by electrons inside the membrane. White blood cells (leukocytes) are also known as "WBC's". White blood cells are made in the bone marrow but they also divide in the blood and lymphatic systems. They are commonly amoeboid (cells that move or feed by means of temporary projections, called pseudopods (false feet), and escape the circulatory system through the capillary beds. Normal range of WBC: $(3\text{-}7)\times10^{3}mm^{3}$.

There are two types of WBC:

Granular leukocytes: different types of granular WBC's are

a. **Basophils**: Basophils store and synthesize histamine which is important in allergic reactions. They enter the tissues and become "mast cells" which help blood flow to injured tissues by the release of histamine.

b. **Eosinophils**: Eosinophils are chemotoxic and kill parasites. Neutrophils are the first to act when there is an infection and are also the most abundant white blood cells.

c. **Neutrophils**: Neutrophils fight bacteria and viruses by phagocytosis which means they engulf pathogens that may cause infection. The life span of a Neutrophil is only about 12-48 hours.

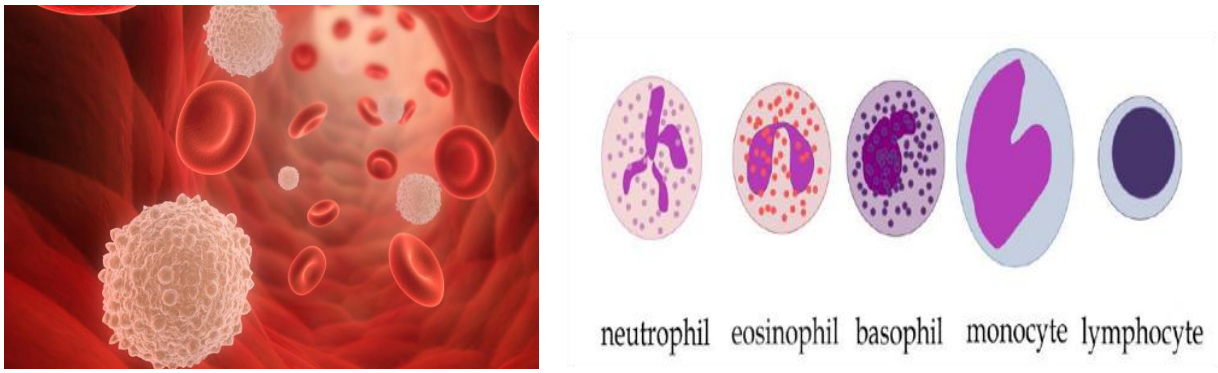


Figure-7: White Blood Cell and Different types of White Blood Cells.

Agranular leukocytes: Two types of agranular WBC are

a. **Monocytes**: Monocytes are the biggest of the white blood cells and are responsible for rallying the cells to defend the body. Monocytes carry out phagocytosis and are also called macrophages.

b. **B-** and **T-cell lymphocytes**: Lymphocytes help with our immune response. There are two Lymphocytes: the B- and T- cell. B-Lymphocytes produce antibodies that find and mark pathogens for destruction. T-Lymphocytes kill anything that they deem abnormal to the body (Ganong, 2003).

1.3.3.3. Platelets

Platelets, also called thrombocytes, are membrane-bound cell fragments. Platelets have no nucleus, they are between one to two micrometers in diameter, and are about 1/10th to 1/20th as abundant as white blood cells. Less than 1% of whole blood consists of platelets. They result from fragmentation of large cells called Megakaryocytes - which are cells derived from stem cells in the bone marrow. Platelets are produced at a rate of 200 billion per day. Their production is regulated by the hormone called Thrombopoietin. The circulating life of a platelet is 8–10 days. The sticky surface of the platelets allows them to accumulate at the site of broken blood vessels to form a clot. This aids in the process of hemostasis ("blood stopping"). Platelets secrete factors that increase local platelet aggregation (e.g., Thromboxane A), enhance vasoconstriction (e.g., Serotonin), and promote blood coagulation (e.g., Thromboplastin). Normal range of platelet: (1000-1600)$\times 10^3 mm^3$ (Ganong, 2003).

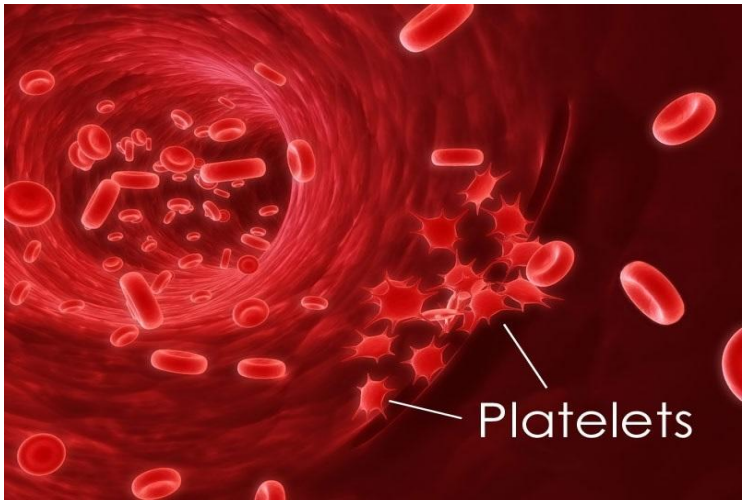


Figure-8: Platelets

Functions:

Blood performs many important functions within the body including:

- Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)
- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins(e.g., blood lipids)
- Removal of waste such as carbon dioxide, urea, and lactic acid
- Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies
- Coagulation, the response to a broken blood vessel, the conversion of blood from a liquid to a semi-solid gel to stop bleeding.
- Messenger functions, including the transport of hormones and the signaling of tissue damage
- Regulation of body pH
- Regulation of core body temperature

1.4. Hepatotoxicity

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

Several factors predispose the liver to xenobiotic toxicity:

- Firstly, for chemicals entering the body via the oral route, anatomical proximity to the GI-tract ensures the liver is the 'first port of call' for ingested xenobiotics.

- Secondly, chemicals and nutrients are not the only substances that enter portal blood as it perfuses the intestines: it also accumulates products of the degradation of intestinal microorganisms such as inflammogenic lipopolysaccharide components of the bacterial cell wall (i.e. endotoxin). Since endotoxin delivery may increase during xenobiotic intoxication, immunological responses to co-absorbed endotoxin can exacerbate the hepato-toxicity of ingested chemicals.

- Thirdly, in addition to entry via the portal circulation, chemicals can access the liver via arterial blood that mixes with venous blood in the hepatic sinusoids. For example, inhaled tobacco constituents that enter via the lungs are efficiently delivered to the liver via the arterial route.

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

1.4.1. Liver

The liver is a vital organ of vertebrates and some other animals. In the human it is located in the upper right quadrant of the abdomen, below the diaphragm. The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemicals necessary for digestion.

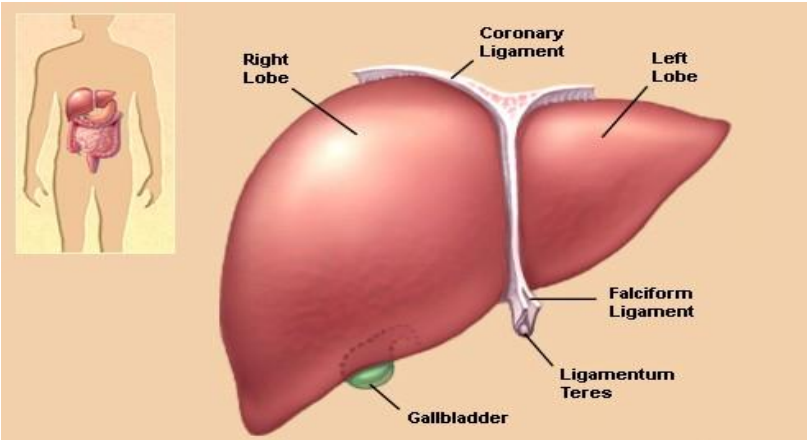


Figure-9: Liver

The liver is a gland and plays a major role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, protein synthesis, hormone production, and detoxification. It is an accessory digestive gland and produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver's highly specialized tissue consisting of mostly hepatocytes regulates 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 (Maton et.al., 1993).

Function

- The liver is considered a gland—an organ that secretes chemicals—because it producesbile, a substance needed to digest fats. Bile's salts break up fat into smaller pieces so it can be absorbed more easily in the small intestine.
- Detoxifies the blood to rid it of harmful substances such as alcohol and drugs
- Stores some vitamins and iron
- Stores the simple sugar glucose
- Converts stored sugar to usable sugar when the body's sugar (glucose) levels fall below normal.
- Breaks down hemoglobin as well as insulin and other hormones
- Converts ammonia to urea, which is vital in metabolism
- Destroys old red blood cells

1.4.2. Liver function tests

Liver function tests (LFTs or LFs) are groups of blood tests that give information about the state of a patient's liver. These tests 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 (Mc.Clatchey, 2002). 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 (Johnston, 1999).

Use of liver function test

- Differential diagnosis of jaundice
- Diagnosis of liver damage
- To asses the extent of liver damage
- To follow the progress of liver

Table-1: Reference value of different protein that distinguish the liver disorders

Parameters	**Reference value**
Total Protein (g/L)	60-80
Albumin (g/L)	33-45
AST (U/L)	<35
ALT (U/L)	<45
ALP (U/L)	54-128
Total Bilirubin (µ mol/L)	0.0-34
Conjugated Bilirubin (µ mol/L)	0.0-3.4

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). 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). This test can help determine if a patient has liver disease or kidney disease, or if the body is not absorbing enough protein. Albumin helps move many small molecules through the blood, including bilirubin, calcium, progesterone, and medications. It plays an important role in keeping the fluid from the blood from leaking out into the tissues.

Decreased blood albumin levels may occur when your body does not get or absorb enough nutrients, such as:

- After weight-loss surgery
- Crohn's disease
- Low-protein diets
- Sprue
- Whipple's disease

Increased blood albumin level may be due to:

- Dehydration
- High protein diet
- Having a tourniquet on for a long time when giving a blood sample (Pratt, et.al. 2010).

Alkaline phosphatase

Alkaline phosphatase (ALP) is an enzyme in the cells lining the biliary ducts of the liver. The test may be done to diagnose liver or bone disease, to check, if treatments for those diseases are working and as part of a routine liver function test.

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
- Rickets

Lower-than-normal ALP levels

- Hypophosphatasia
- Malnutrition
- Protein deficiency
- Wilson's disease (Martin, 2011).

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. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage. This test is used to determine if a patient has liver damage (Nyblom et.al., 2004).

An increase in ALT levels may be due to:

- Cirrhosis (scarring of the liver)
- Death of liver tissue (liver necrosis)
- Hepatitis

- Lack of blood flow to the liver (liver ischemia)
- Liver tumor or cancer
- Medications that are toxic to the liver
- Pancreatitis (swollen and inflamed pancreas)

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 GPT level is tested to look for and evaluate damage to the liver. It is also measured to check medical treatments that may lead to liver inflammation.

SGPT levels may be higher than normal also if:

- drink too much alcohol
- chronic liver infection or inflammation
- gallbladder infection and inflammation, such as may caused by gallstones
- 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 cholesterol lowering agent, antifungal medicines, some narcotics and barbiturates, methotrexate, acetaminophe salicylates (aspirin) (Pratt, 2010).

Chapter-2
Plant Introduction

2.1. Plant information

Trapa bispinosa which belongs to the family Trapaceae is a small herb well known for its medicinal properties and is widely used worldwide.The medicinal values of the whole herb and fruit have long been recognized in folklore medicine as a cure for various diseases (Prajapati ND et.al.,2003).

2.1.1. Scientific name

Trapa bispinosa

2.1.2. Local Name

Paniphal

Singhara

Singra kata

2.1.3. Common name

English: Water chest nut

Bengali: Paniphal

Gujarati: Singora

Hindi: Simghara

Sanskrit: Smgtakah

Tamil: Chimkhara

Telugu: Kubjakamu

Urdu: Singhara.

(Prajapati ND, 2005;Ghani N ,2002; Kabeeruddin M et. al., 2010)

2.1.4. Synonym

- *Trapa bispinosa* var. *iinumai* Nakano
- *Trapa chinensis* Lour.
- *Trapa cochinchinensis* Lour.
- *Trapa congolensis* Vassiliev
- *Trapa natans* Thunb.
- *Trapa natans* var. *bispinosa* (Roxb.)Makino

2.1.5. Taxonomic position:

Kingdom: Plantae

Subkingdom: Tracheobointa

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Rosidae

Order: Myrtales

Family: Trapaceae

Genus: *Trapa*

Species*:* *Trapa bispinosa* (Itis 2012)

2.2. Description:

Trapa bispinosa is an herbaceous, floating-leaf aquatic species that often grows in water around 60 cm deep (PFAF, 2000). The floating leaves are arranged in a rosette, with leathery upper leaves up to 5 cm wide and broadly rhomboid, triangular, deltoid or broadly ovate (Hummel and Kiviat, 2004). The leaves are sharply serrate, with conspicuous venation and short, stiff hairs. The species also produces submersed leaves that are strikingly morphologically different (Bitonti et. al., 1996). The submersed leaves are alternate, finely divided, and can grow up to 15 cm long (Mehrhoff et. al.,2003). The petioles of the floating leaves have a spongy section that allows for the flotation of the leaf rosette, and each stem may produce several rosettes (Hummel and Kiviat, 2004). The plant also has white flowers with four 8 mm-long petals and four green sepals. The fruit is a horned nut-like structure that develops underwater and is approximately 3 cm wide (Mehrhoff et.al., 2003). Single flowers are produced in axils of floating leaves (Hummel and Kiviat, 2004). The stem of the plant is flexible and from 1 to 5 m long, nodes of the stem have slender linear roots, while the plant is anchored in the sediment by the lower roots that emerged from the propagating seed hull (Hummel and Kiviat, 2004).

Figure-10 : Whole plant and fruit of *Trapa bispinosa*.

2.3. Geographical Distribution

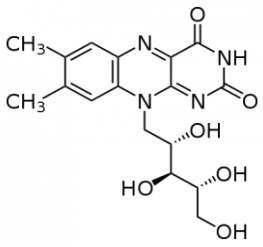
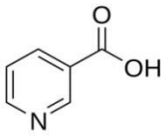
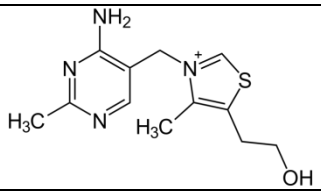
The genus *Trapa* is cultivated worldwide for the harvest of its large, nutritious nut. It currently occupies a wide yet discontinuous range across Europe, Asia, and Africa, and has been introduced to North America and Australia. The variety *T. natans* var. *natans*, with its four spined nut is widely distributed in Eurasia, Africa and the northeastern United States, whereas *T. natans* var. *bispinosa* (also known as *T. bicornis*, *T. bicornuta*, or *T. japonica)*, a two-spined variety, grows in China, Japan, India and Southeast Asia (Hummel and Kiviat, 2006). In Bangladesh, it is found in Dhaka , Mymensing , Rajshahi and other districts,in ponds and ditches.

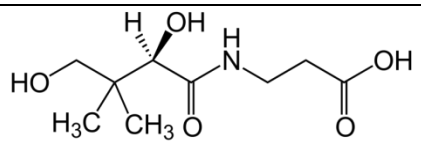
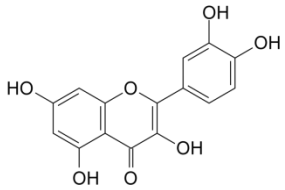
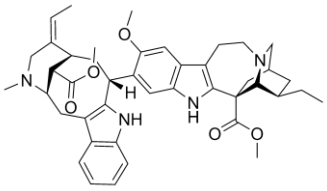
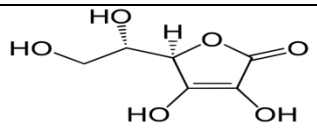
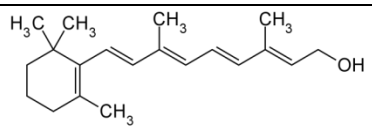
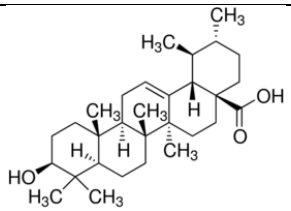
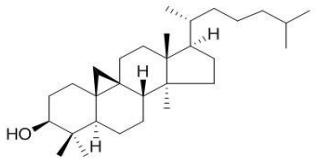
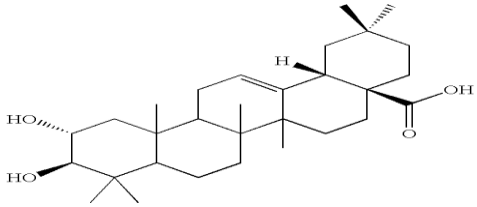
2.4. Chemical composition

Trapa contains a great quantity of non-nutritional antioxidants, such as flavoniods, flavone and total phenolic contents. Flavonoids are present in plant tissues, such as fruits, vegetables, nuts, seeds and leaves, in relatively high concentrations. Flavonoids act as natural antioxidants. Phytochemical screening of seed extract of T. natans fruits reveals the presence of carbohydrates, saponins, phytosterols, fixed oils and fat while the pericarp extract of the fruits of T. natans revealed the presence of tannins, flavonids and glycosides (Guo JT et.al.,2001;Havsteen,1983).

From the hydroalcoholic extract Trapa pseudoincisa Nakai, three compounds were isolated. The chemical structures of the compounds were determined as cycloeucalenol , ursolic acid , and 2β,3α,23-trihydroxyurs- 12-en-28-oic acid (Song MC et.al.,2007).

Table-2: Different Chemical Constituents of *Trapa bispinosa.*

Name of Constituents	Chemical Structure
Riboflavin (vitamin B2)	
Nicotinic acid/Niacin (vitamin B3)	
Thiamine (vitamin B1)	

Pantothenic acid (vitamin B5)	
Flavonoids	
Alkaloids	
Arscobic acid (vitamin C)	
Retinol (vitamin A)	
Triterpenoids (ursolic acid)	
Triterpenoids (cycloeucalenol)	
(2β, 3α)-2,3-Dihydroxy-urs-12-en-28-oic acid	

2.5. Medicinal Uses

It is used in many Ayurvedic preparations as nutrient, appetizer, astringent, diuretic, aphrodisiac, tonic, cooling and antidiarrheal agents. It is also useful in lumbago, sore throat, bilious affections, bronchitis, fatigue and inflammation. The medicinal values of the whole herb and fruit have long been recognized in folklore medicine as a cure for various diseases(Rahman MM et.al.,2001). The whole herb has been reported for hepatoprotective activity, antimicrobial activity, antibacterial activity, antitumor activity, antioxidant activity and free radical scavenging activity. Further, the fruits have been used as intestinal astringent, aphrodisiac, antiinflammatory, antileprotic agent and in urinary discharges, fractures, sore throat, bronchitis and anemia(Kirtikar KR et.al.,2006). In addition to this, the juice of the fruit has been used for diarrhea and dysentery. Fruits are also being used in making liniments for the cure of rheumatism, sores and sunburn. It is also said to have cancer-preventing properties. Stem juice is used in Ophthalmic preparations (Karmakar et al., 2011).

2.6.Pharmacological activities

The *Trapa* species have multiple pharmacological actions. In general, anti-microbial, anti-inflammatory, anti-diadetic activity, analgesic, antioxidant even in central nervous system. In this section, our aim is to highlight the pharmacological experiments and studies reported with species of the genus *Trapa.*

Analgesic Activity

Analgesic activity of the methanolic extract of the T. bispinosa root at a dose of 200 mg/kg and 400 mg/kg was evaluated against the standard drug pentazocine at a dose of 30 mg/kg. Adult Swiss albino mice of either sex of six numbers in each group were undertaken for study and evaluated by tail flick and tail immersion method.

Both doses of T. bispinosa roots methanolic extract were found to produce significant analgesic activity. In tail flick method, the extract at 200 mg/kg showed significant activity after 45 minutes, but in tail immersion method, the extract showed significant activity at all tested dose levels after 30-minute interval. The results showed significant analgesic activity against stimuli(Anuj k Agrahari et al., 2010).

Antimicrobial

The methanolic extract of this plant at the concentration of 200 µg/disc showed a more potent antimicrobial activity against Gram positive (*Bacillus subtilis, B. cereus, B. megaterium, Staphylococcus aureus* and *Staphylococcus β-haemolyticus*) and Gram negative (*Escherichia coli, Klebsiella, Pseudomonas aeruginosa, Shigella dysenteriae, Shigella flexneri, Shigella sonnei, Shigella boydii, Salmonella typhi* A and *Salmonella typhi* B-56) bacteria than the activity shown by ethyl acetate, chloroform and petroleum ether extracts. The most significant cytotoxic

activity in the brine shrimp lethality assay was observed for the chloroform extract(Agarwal et al.,2011).

Antibacterial Activity

Antibacterial activities of the fruit extract of two varieties (green and red) of water chestnut by the disc diffusion method from methanol extract were studied. The extract of red variety of water chestnut showed high antibacterial potential (31 mm) against Bacillus subtilis with the concentration of 600 micron. On the other hand, green variety showed highest antibacterial activities (12 mm) against both Staphylococcus aureus and Shigellasonnei with the concentration of 600 microgram Kanamycin used as standard. In this disc diffusion assay, the methanol extract of red variety was found to have a significant antibacterial efficiency compared to the extract of green variety of water chestnut. These findings pinpoint the efficiency of these extracts to inhibit microbial growth (Mohammad Razvy A et al. 2011).

Antidiabetic Activity

To evaluate the antidiabetic activity of methanol extract of T. natans fruit peels (METN) in Wistar rats, the effect of METN on oral glucose tolerance and its effect on normoglycemic rats were studied. Diabetes was induced in rats by single intraperitonial injection of streptozotocin. Three days after STZ induction, the hyperglycemic rats were treated with METN orally at the dose of 100 and 200 mg/kg body weight daily for 15 days.

METN at the dose of 100 and 200 mg/kg orally significantly and dose dependently improved oral glucose tolerance exhibited hypoglycaemic effect in normal rats and antidiabetic activity in STZ-induced diabetic rats by reducing and normalizing the elevated fasting blood glucose levels as compared to those of STZ control group (Das Prashanto K et al. 2011).

Antiinflammatory activity

Fruits of *Trapa natans* L. var. *bispinosa*, commonly known as Shingoda, were reported to be potential antiinflammatory agent in traditional literatures. Antiinflammatory activity was performed by using Carrageenan induced hind paw edema model. The aqueous extract of pericarp had shown significant anti-inflammatory activity by decreasing paw volume on the 3rd and the 5th hour, while the aqueous extract of seed showed significant antiinflammatory activity by decreasing the paw volume at the 5th hour only(Patel SA et al.,2011).

Antioxidant

Aqueous extract of *Trapa natans* L. fruits had shown potential *in vitro* antioxidant activity. The extract was found to contain a large amount of polyphenols and also exhibited an immense reducing ability. The total content of phenolics, flavonoids and tannin compounds was estimated as 63.81 mg of gallic acid equivalents/g of dry material, 21.34 mg of rutin equivalents/g of dry

material and 17.11 mg of total tannin equivalent/g of dry material, respectively. Reducing power and inhibition of OH radicalinduced bovine serum albumin (BSA) oxidation were also determined. The data obtained from the study suggested that the aqueous extract of *Trapa natans* L. fruit rind had significant antioxidant activity against free radicals.9, 32, 38 The effect of hydroaccoholic extract of *T. bispinosa* (TB) was studied on fluorescence product and biochemical parameter like peroxidation catalase activity and glutathione peroxidase activity in brain of female Albino mice. Ageing was accelerated by the treatment of 0.5 ml of 5% D–glucose for 15 days. This resulted in increased fluorescence product showed an increase in lipid peroxidase and decrease the antioxidant enzyme like glutathione peroxides and catalase in cerebral cortex. After cotreatment with hydroalcoholic extract of TB (500 mg/kg) there was decrease in fluorescence product in cerebral cortex. Moreover, TB inhibited increase lipid peroxidation and restores glutathione peroxidase and catalase activity in cerebral cortex as compare to ageing accelerated control group. Thus the extract was found to be effective as an antioxidative agent which could reverse D-galactose induced ageing changes resulting due to oxidative damage(kar D et al.,2004).

Antiulcer Activity

The antiulcer activity of the fruits of Trapa bispinosa was studied on Wistar rats. The antiulcer activity of 50% ethanolic extract at two dose levels was evaluated by using pyloric ligation and aspirin plus pyloric ligation models. The tests extract revealed significant antiulcer activity, which might be due to increase in total carbohydrate content and alter state of mucosal barrier of the stomach. The results indicate that the ethanolic extract of fruits of Trapa bispinosa is endowed with potential antiulcer activity (Kar DM et al. 2010).

Neuroprotective Activity

Effect of hydroalcoholic extract of Trapa bispinosa was studied on fluorescence product and biochemical parameters like lipid peroxidation, catalase activity, and glutathione peroxidase activity in brain of female albino mice. Ageing was accelerated by the treatment of 0.5 mL 5%D-galactose for 15 days. This resulted in increased fluorescence product, increased lipid peroxidation and decreased antioxidant enzyme like glutathione peroxidase and catalase in cerebral cortex. After cotreatment with hydroalcoholic extract of Trapa bispinosa (500 mg/kg, p.o) there was decrease in fluorescence product in cerebral cortex. Moreover, T. bispinosa inhibited increased lipid peroxidation and restored glutathione peroxidase and catalase activity in cerebral cortex as compared to ageing accelerated control group (Ambikar DB et al. 2010).

Noortropic

TB extract showed significant facilitatory effect on aversively investigated for its noortropic activity using various experimental paradigms of learning and memory, *viz.* transfer latency (TL) on elevated plus-maze, passive avoidance response (PAS) and object recognition test. The investigation reported that TB 500 mg/kg significantly reduced the TL on 2nd and 9th day while

TB 250 mg/kg was found effective on 9th day. TB 250 and 500 mg/kg significantly increased the step down latency in the PAS at acquisition and retention test. Moreover the TB (250 & 500 motivated learning and memory in mice as well as improvement of memory in absence of cognitive deficit. From the above experiment it was proved that the hydroalcoholic extract of TB (Ambikar DB et al. 2010).

Imunomodulatory

Aqueous extract of fruits of *T. bispinosa* (TBAE) showed promising immunomodulatory function. The immunomodulatory effect was assessed in rats against sheep red blood cells (SRBC) as antigen by studying cell-mediated delayed type hypersensitivity reaction (DTH), humeral immunity response and percent change in neutrophil count. Macrophage phagocytosis assay was carried out by carbon clearance method in mice. Oral administration of TBAE dependently increased immunostimulatory responses. Delayed type hypersensitivity reaction was found to be augmented significantly ($p<0.05$) by increasing the mean foot pad thickness at 48 hr and production of circulatory antibody titre (humoral antibody response) was significantly ($p<0.05$) increased in response to SRBC as an antigen. In addition, immunostimulation was counteracted by upregulating macrophage phagocytosis in response to carbon particles. Immunostimulatory property of TBAE further **c**onfirmed by elevating neutrophil counts significantly ($p<0.01$), as compared to control. The results of the present study suggested that the aqueous extract of fruits of *T. bispinosa* could stimulate the cellular and humoral response in animals(Makare N et al.,2001).

Cytotoxic study:

Brine shrimp lethality bioassay was carried out to investigate the cytotoxicity of plant extracts (Meyer et.al) . It was carried out to investigate the cytotoxicity of the extract. Brine shrimps (*Artemia salina*) were hatched using brine shrimp eggs in a conical shaped vessel (1L), filled with sterile artificial seawater (prepared by using sea salt 38 g/L and adjusted pH 8.5) under constant aeration for 48h. After hatching, active nauplii free from egg shells were collected from brighter portion of the hatching chamber and used for the assay. Ten nauplii were drawn through a micropipette and placed in each test tube containing 4.5 mL of brine solution. In each experiment, 0.5 mL of the extract was added to 4.5 mL of brine solution and maintained at the ambient room temperature for 24 h and surviving nauplii were counted. For the investigations test solution of the extract was prepared by dissolving 20 mg of the extract in 1 mL of pure dimethyl sulfoxide (DMSO). 500µl of solution was taken in test tubes each containing 500 µl of simulated seawater. Stock solution having the concentration 1mg/mL was obtained by adding 9mL of simulated sea water in the test tube. A series of solutions of varying concentrations were prepared from the stock solution by serial dilution method. Tests were conducted along with negative control (DMSO treated), and different concentrations (1 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, 50 µg/mL, 100 µg/mL, 200 µg/mL and 500 µg/mL) of the leaves extract of *T.bispinosa*in a set of two tubes per dose (Wahed TB et al., 2001).

Chapter-3
Materials and Methods

3.1. Plant Preparation

3.1.1 Collection of plant

The plant was collected from Rajshahi district of Bangladesh. A voucher specimen (Accession number: 38716) 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-11: Herbarium sheet of *Trapa bispinosa.*

3.1.2. Preparation of plant extraction

The fruits 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 *Trapa bispinosa* 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-12: Rotary evaporator & crude extract in a bottle

3.2. 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-13: *Swiss albino* Mice

3.3. 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.

3.4. Drugs and Chemicals

3.4.1. 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.

3.4.2. Standard Drug

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

3.5. CNS Activity Test

3.5.1. 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

3.5.2. Chemical Agents Used in CNS activity Test

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

3.5.3. 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.

3.5.4. Doses Used in CNS Activity Test of the Extract

I. Open Field Test:

Methanolic extracts of *Trapa bispinosa* at a dose of 200mg/kg, 400mg/kg & 600mg/kg of the crude extract are administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses.

II. . Hole Board Test:

Methanolic extracts of *Trapa bispinosa* at a dose of 200mg/kg, 400mg/kg & 600mg/kg of the crude extract are administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses.

3.5.5. 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.

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 ten 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 *Trapa bispinosa* at the doses of 200,400 & 600mg/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-14: Open Field Test

Hole Board Test:

The hole board represents a combination of a hole board, originally designed to investigate explorative motivation in rodents (Lister, 1990) and later on modified to evaluate cognitive functions (Ohl and Fuchs, 1999; Ohl et. al., 1998). The hole board itself consisted of a total of 16 holes, each 3 cm in diameter, were presented to the mouse in a flat space of 25 square centimeters. This experiment was carried out by the following method of Boisser and Simon, 1964. The animals were divided into negative control and test groups containing six mice in each group. Negative control group received vehicle (5% CMC solution) at a dose of 10 mg/kg body

weight orally. The test groups received extracts of *Trapa bispinosa* at the doses of 200 mg/kg,400 mg/kg & 600mg/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

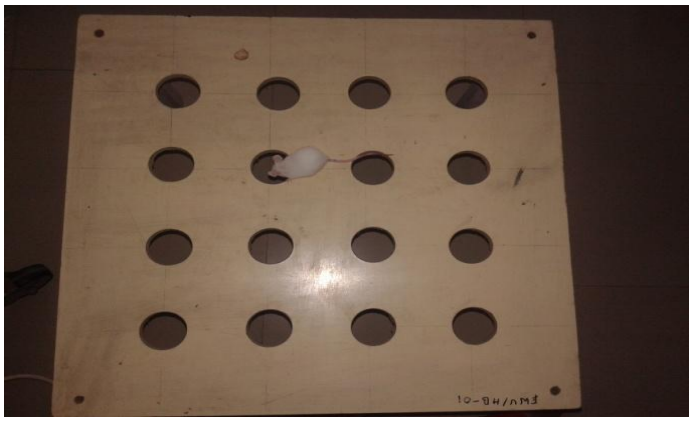


Figure-15: Hole Board Test

3.6. Toxicity Test

3.6.1 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

3.6.2 Chemical Agents Used Toxicity Test

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

3.6.3 Doses Used for Toxicological Activity of the Extract

i. Acute Toxicity Test:

Methanolic extracts of *Trapa bispinosa* at a dose of 2000mg/kg, 4000mg/kg and 6000mg/kg were administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses.

ii. Chronic Toxicity Test:

Methanolic extracts of *Trapa bispinosa* at a dose of 200, 400 mg/kg & 600mg/kg are administered orally. 5% CMC is used as a vehicle with plant methanolic extract for preparing different doses.

3.6.4 Methods for Toxicity Test

Acute Toxicity Test

The acute toxicity of in Swiss albino mice was studied as reported method. Each extract were given to three groups (n = 10) of mice at 2000 and 4000, 6000 mg/kg body weight, orally. The treated animals were kept under observation for 3 days, for mortality and general behavior (Paul, et.al. 2012).

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, 600 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 90th 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).

Figure-16: Mice Organ and blood

3.6.5. 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)

3.6.6. 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)

3.6.. 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- 4
Result and Discussion

4.1.CNS Activity Test of Methanolic Extract of *Trapa bispinosa*

4.1.1. Open Field Test

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

Total Number of Peripheral locomotion, Central locomotion, Leaning, Rearing, Grooming, Defecation count.

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

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

- ✓ The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 121.50±1.05, at 30 min 121±2.62 ,at 60 min 118±0.56, at 90 min119.83±0.79 and at 120 min 121.83±1.10 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of central locomotion count is followed with a mean value of at 0 min 22.67±1.22, at 30 min 20.67±0.91,at 60 min 21.50±0.92, at 90 min 21.0±0.57and at 120 min 121.83±1.10 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of leaning count is followed with a mean value of at 0 min 20.33±0.76, at 30 min 21.16±2.13,at 60 min 21.33±0.80, at 90 min 17.67±0.76and at 120 min 21.33±0.80 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of rearing count is followed with a mean value of at 0 min 0.67±0.33, at 30 min 0.67±0.33,at 60 min 0.16±0.16, at 90 min 0.67±0.21 and at 120 min 1.00±0, 25 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of grooming count is followed with a mean value of at 0 min 0.50±0.22,at 30 min 0.50±0.22, at 60 min 0.50±0.34, at 90 min 0.67±0.21 and at 120 min 0.66±0.21 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of defecation count is followed with a mean value of at 0 min 0.67±0.21, at 30 min 0.67±0.21, at 60 min 0.50±0.22, at 90 min 0.83±0.30and at 120 min 0.83±.16 (Mean ±SEM) during 2 minutes observation.

Positive Control Group (Diazepam, 1mg/kg)

This group of mice receives the standard drug Indomethacin of 10mg/kg orally.

- ✓ The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 111.67±1.3, at 30 min 69.0±1.06, at 60 min 50.0±1.54,at 90 min 26.83±1.19, and at 120 min 15.0±1.41, (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of central locomotion count is followed with a mean value of at 0 min 19.16±1.01, at 30 min 9.83±0.79,at 60 min 4.16±0.47,at 90 min 3.00±0.51and at 120 min 2.0±0.25 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of leaning count is followed with a mean value of at 0 min 18.33±0.42,at 30 min 9.0±0.57,at 60 min 7.5±0.42,at 90 min 5.0±0.36and at 120 min 2.83±0.54 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of rearing count is followed with a mean value of at 0 min1.0±0.36,at 30 min 0, at 60 min 0, at 90 min 0 and at 120 min 0 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of grooming count is followed with a mean value of at 0 min 1.0±0.25,at 30 min 0, at 60 min 0, at 90 min 0 and at 120 min 0 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of defecation count is followed with a mean value of at 0 min 0.83±0.30,at 30 min 1.0±0.36,at 60 min 1.16±0.30,at 90 min 0.33±0.21and at 120 min 0.50±0.22 (Mean ±SEM) during 2 minutes observation.

Test Group-1 (Plant Extract, 200mg/kg)

This test group of mice receives the plant extract of 200 mg/kg orally.

- ✓ The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 114.5±1.73,at 30 min 86.3±1.20,at 60 min 66.5±1.97,at 90 min 52.5±1.65and at 120 min 32.0±1.82 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of central locomotion count is followed with a mean value of at 0 min 13.2±0.79,at 30 min 6.7±0.98,at 60 min 6.0±0.93,at 90 min 3.7±0.56and at 120 min 2.3±0.21 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of leaning count is followed with a mean value of at 0 min 15.5±0.99,at 30 min 10.8±0.48,at 60 min 8.2±0.48,at 90 min 5.8±0.47and at 120 min 3.3±0.21 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of rearing count is followed with a mean value of at 0 min 0.81±0.83,at 30 min 1.65±1.05,at 60 min 1.20±1.02,at 90 min 1.0±1.0 and at 120 min 0 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of grooming count is followed with a mean value of at 0 min 2.0±0.70,at 30 min 1.54±0.65,at 60 min 0.31±0.33,at 90 min 0.63±0.33and at 120 min 0(Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of defecation count is followed with a mean value of at 0 min 0.15±0.08,at 30 min 0, at 60 min 0, at 90 min 0 and at 120 min 0 (Mean ±SEM) during 2 minutes observation.

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

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

- ✓ The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 96.2±1.96,at 30 min 78.2±1.47,at 60 min 59.3±0.84,at 90 min 44.5±1.96and at 120 min 28.8±1.58 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of central locomotion count is followed with a mean value of at 0 min 12.7±0.67,at 30 min 6.8±0.87,at 60 min 5.5±0.76,at 90 min 3.0±0.37and at 120 min 2.2±0.17 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of leaning count is followed with a mean value of at 0 min 13.8±0.87,at 30 min 9.0±0.37,at 60 min 6.8±0.31,at 90 min 5.0±0.45and at 120 min 2.8±0.30 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of rearing count is followed with a mean value of at 0 min 0, at 30 min 0, at 60 min 0.73±0.34,at 90 min 0 and at 120 min 0.50±0.50 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of grooming count is followed with a mean value of at 0 min 1.73±0.50,at 30 min 0.33±0.33,at 60 min 0, at 90 min 1.14±0.64 and at 120 min 0(Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of defecation count is followed with a mean value of at 0 min 0.30±0.12,at 30 min 0.33±0.21,at 60 min 0.16±0.16,at 90 min 0 and at 120 min 0 (Mean ±SEM) during 2 minutes observation.

Test Group-3 (Plant Extract, 600mg/kg)

These groups of mice receive the plant extract of 600 mg/kg orally.

- ✓ The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 89.7±1.82,at 30 min 68.3±1.82,at 60 min 52.0±1.15,at 90 min 36.0±1.65and at 120 min 24.2±1.40 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of central locomotion count is followed with a mean value of at 0 min 8.7±1.02,at 30 min 5.7±0.61,at 60 min 4.7±0.56,at 90 min 2.5±0.22and at 120 min 1.8±0.30 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of leaning count is followed with a mean value of at 0 min 10.8±0.60,at 30 min 7.7±0.33,at 60 min 5.7±0.33,at 90 min 3.3±0.3and at 120 min 2.0±0.26 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of rearing count is followed with a mean value of at 0 min 0, at 30 min 0, at 60 min 0.63±0.24,at 90 min 0 and at 120 min 0 (Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of grooming count is followed with a mean value of at 0 min 0, at 30 min 0.21±0.03,at 60 min 1.16±0.74,at 90 min 0 and at 120 min 0(Mean ±SEM) during 2 minutes observation.

- ✓ The observed total number of defecation count is followed with a mean value of at 0 min 0.33±0.33,at 30 min 0.33±0.21,at 60 min 0, at 90 min 0 and at 120 min 0 (Mean ±SEM) during 2 minutes observation.

Table-3: CNS Activity of plant extract of *Trapa bispinosa* by Open Field Test (Central Locomotion) in Mice.

Groups	Dose	No. of Central Locomotion				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	**10ml/kg**	20.0±1.32	11.33±1.26	14.5±1.98	12.33±1.52	13.5±1.43
Crude extract of *Trapa bispinosa*	**200mg/kg**	13.2±0.79	6.7±0.98	6.0±0.93	3.7±0.56	2.3±0.21
Crude extract of *Trapa bispinosa*	**400mg/kg**	12.7±0.67	6.8±0.87	5.5±0.76	3.0±0.37	2.2±0.17
Crude extract of *Trapa bispinosa*	**600mg/kg**	8.7±1.02	5.7±0.61	4.7±0.56	2.5±0.22	1.8±0.30
Positive control Diazepam	**1mg/kg**	20.67±1.05	9.5±0.76	6.17±0.6	4.17±0.6	3.33±0.42

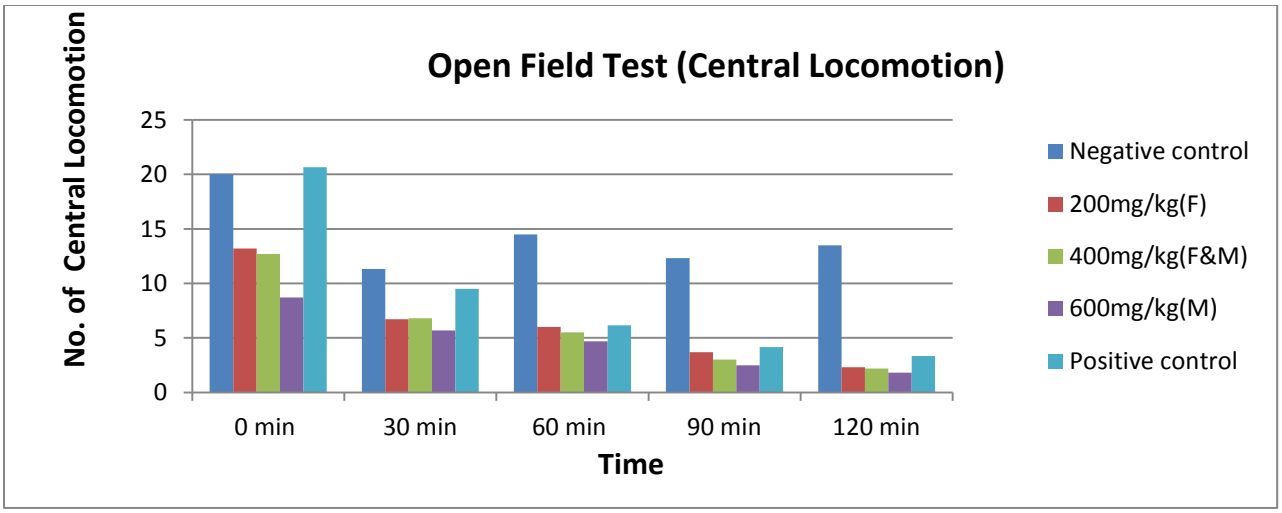


Figure-17: Graphical Presentation of CNS Activity of plant extract of *Trapa bispinosa* by Open Field Test (Central Locomotion) in Mice.

Table-4: CNS Activity of plant extract of *Trapa bispinosa* 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 *Trapa bispinosa*	200mg/kg	114.5±1.73	86.3±1.20	66.5±1.97	52.5±1.65	32.0±1.82
Crude extract of *Trapa bispinosa*	400mg/kg	96.2±1.96	78.2±1.47	59.3±0.84	44.5±1.96	28.8±1.58

Crude extract of *Trapa bispinosa*	**600mg/kg**	89.7±1.82	68.3±1.82	52.0±1.15	36.0±1.65	24.2±1.40
Positive control Diazepam	**1mg/kg**	121.83±1.1	69.33±1.12	53.0±1.81	35.67±1.17	27.83±1.72

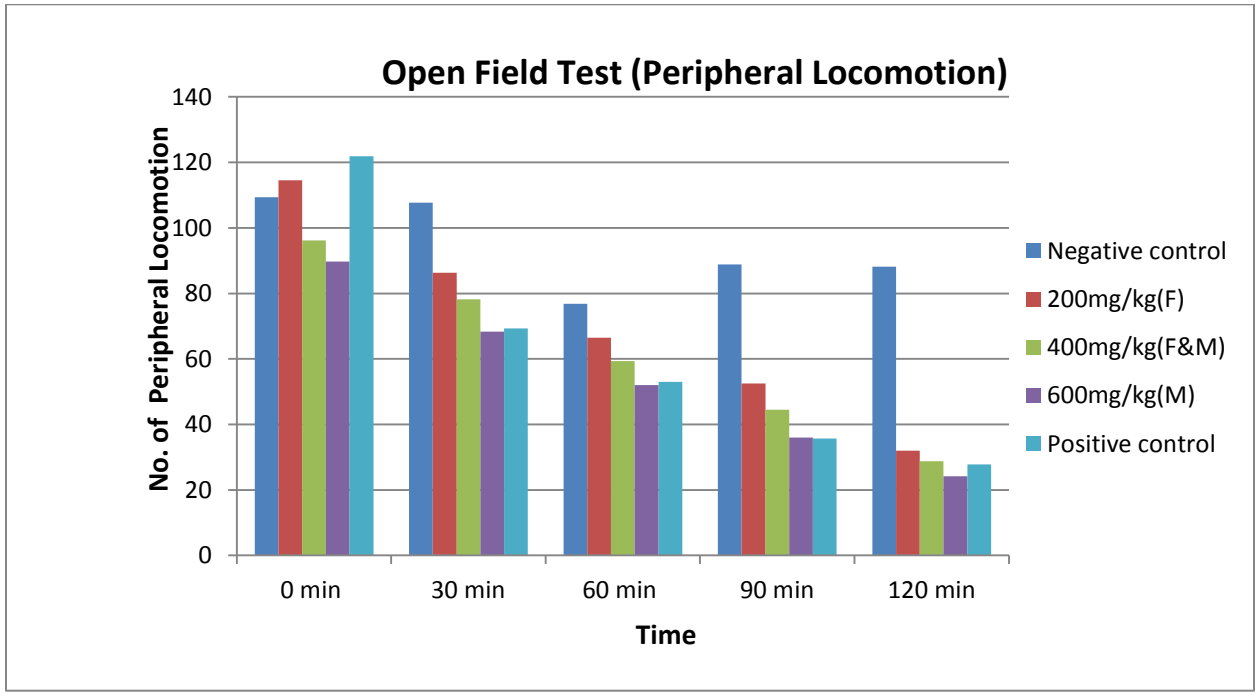


Figure-18: Graphical Presentation of CNS Activity of plant extract of *Trapa bispinosa* by Open Field Test (Peripheral Locomotion) in Mice.

Table-5: CNS Activity of plant extract of *Trapa bispinosa* by Open Field Test (Leaning) in Mice.

Groups	Dose	No. of Leaning Locomotion				
		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 *Trapa bispinosa*	200mg/kg	15.5±0.99	10.8±0.48	8.2±0.48	5.8±0.47	3.3±0.21
Crude extract of *Trapa bispinosa*	400mg/kg	13.8±0.87	9.0±0.37	6.8±0.31	5.0±0.45	2.8±0.30
Crude extract of *Trapa bispinosa*	600mg/kg	10.8±0.60	7.7±0.33	5.7±0.33	3.3±0.3	2.0±0.26
Positive control Diazepam	1mg/kg	22.17±1.08	8.83±0.31	6.17±0.31	4.33±0.33	3.17±0.48

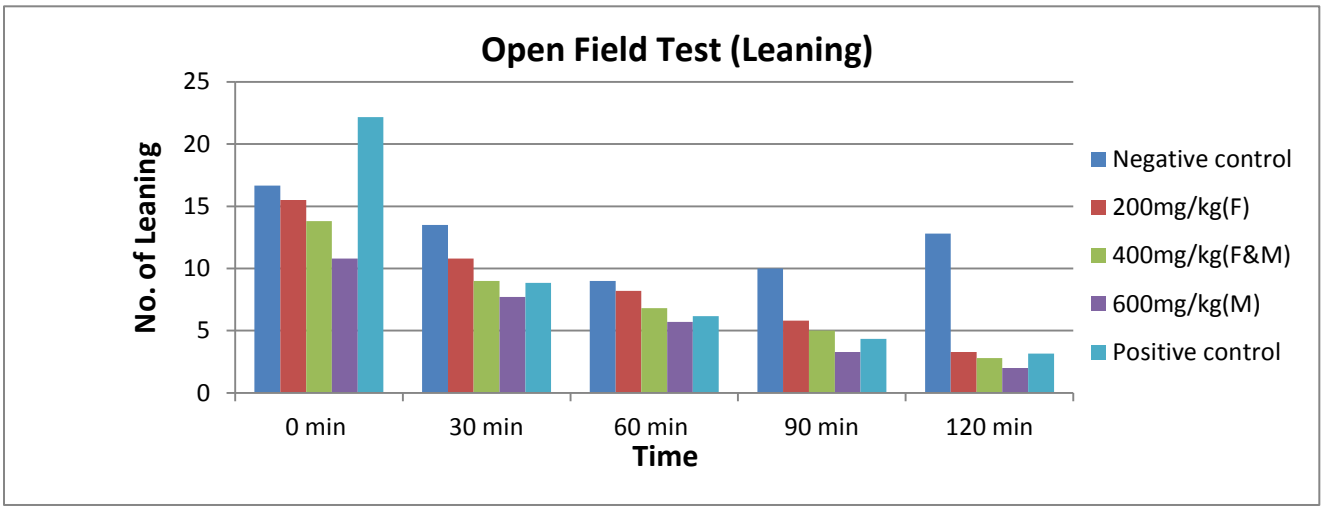


Figure-19: Graphical Presentation of CNS Activity of plant extract of *Trapa bispinosa* by Open Field Test (Leaning) in Mice.

4.1.2.Hole Board Test

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

Total Number of Head Poking Head Dipping and count

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

This group of animals only received vehicle (5% CMC) 10 ml/kg orally. The observed total number of head poking is with a mean value of 68.5±1.48 (Mean ±SEM) and head dipping with mean value of 25.17±1.01 (Mean ±SEM) during 5 minutes observation after 30 min of administration.

Positive Control Group (Diazepam, 1mg/kg)

This group of mice receives the standard drug Diazepam of 1mg/kg orally. The observed total number of head poking is with a mean value of 29.83±1.01 (Mean ±SEM) and head dipping with mean value of 15.67±0.67 (Mean ±SEM) during 5 minutes observation after 30 min of administration.

Test Group-1 (Plant Extract, 200mg/kg)

This test group of mice receive the plant extract of 200 mg/kg orally. The observed total number of head poking is with a mean value of 54.33±0.88 (Mean ±SEM) and head dipping with mean value of (Mean ±SEM) during 5 minutes observation after 30 min of administration.

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

This group of mice receive the plant extract of 400 mg/kg orally. The observed total number of head poking is with a mean value of 33.8±1.35 (Mean ±SEM) and head dipping with mean value of 14.0±0.58 (Mean ±SEM) during 5 minutes observation after 30 min of administration.

Test Group-3 (Plant Extract, 600mg/kg)

This group of mice receive the plant extract of 600 mg/kg orally. The observed total number of head poking is with a mean value of 30.0±1.63 (Mean ±SEM) and head dipping with mean value of 10.7±0.49 (Mean ±SEM) during 5 minutes observation after 30 min of administration.

Table-6: CNS Activity of plant extract of *Trapa bispinosa* by Hole Board Test in Mice.

Group	Treatment	Dose	No. of Head Poking	No. of Head Dipping
Negative control	**5% CMC**	**10 ml/kg**	68.5±1.48	25.17±1.01
Group-1	**Crude extract of *Trapa bispinosa***	**200 mg/kg**	40.7±1.43	19.8±0.70
Group-2	**Crude extract of *Trapa bispinosa***	**400 mg/kg**	33.8±1.35	14.0±0.58
Group-3	**Crude extract of *Trapa bispinosa***	**600 mg/kg**	30.0±1.63	10.7±0.49
Positive control	**Diazepam**	**1 mg/kg**	29.83±1.01	15.67±0.67

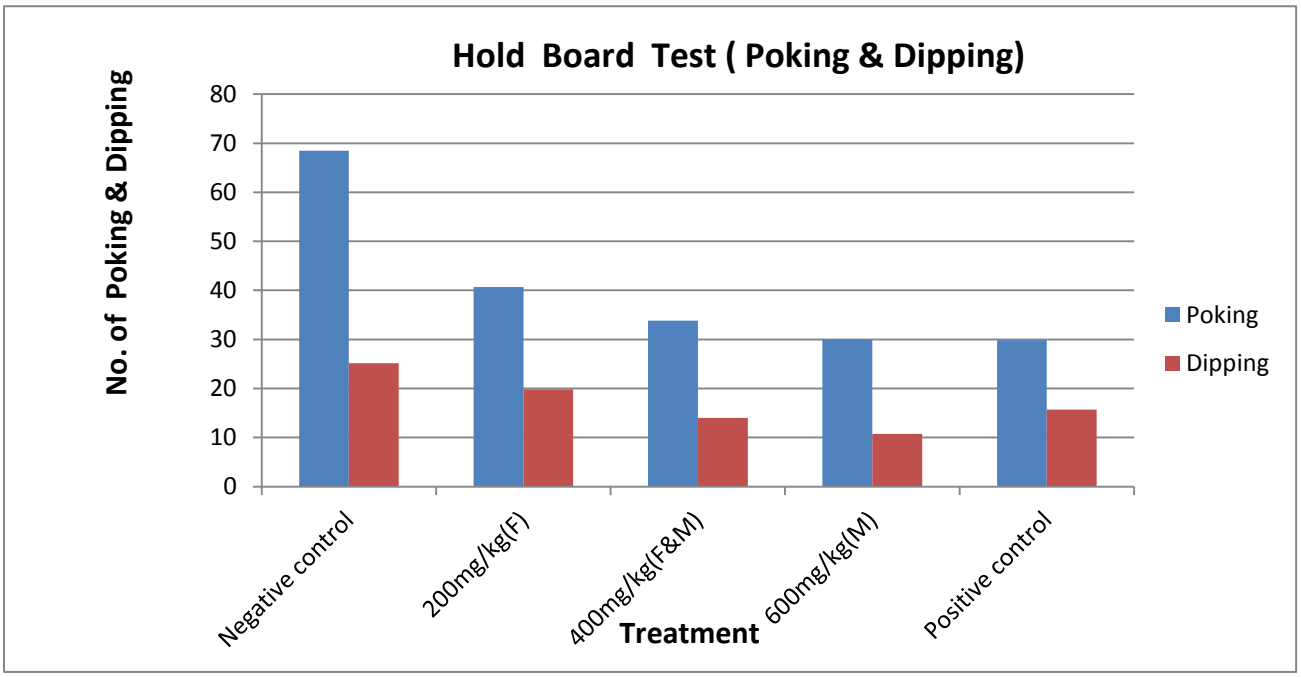


Figure-20: Graphical Presentation of CNS Activity of plant extract of *Trapa bispinosa* by Hole Board Test in Mice.

4.2. Acute and Chronic Toxicity Test

4.2.1. Acute toxicity

For 3 days observation no death was observed till the end of the study.

4.2.2. Chronic Toxicity Test

CBC (Count Blood Cell) Test, Biochemical Test

Drug dose 200,400 and 600 mg/kg (CBC & Biochemical Test)

In the chronic study of methanolic extract of *Trapa bispinosa* at a dose (200,400,600 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 *Trapa bispinosa* 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 *Trapa bispinosa*.

Table-7: Effect of methanolic extract of *Trapa bispinosa* on body weight in mice

Treatment Group	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
***Trapa bispinosa* 200mg/kg**	**Female**	24.5±0.80	27.9±1.45	0
***Trapa bispinosa* 400mg/kg**	**Female & Male**	25.7±1.42	30.2±1.27	2

***Trapa bispinosa* 600mg/kg**	**Male**	26.2±0.85	36.3±3.22	5

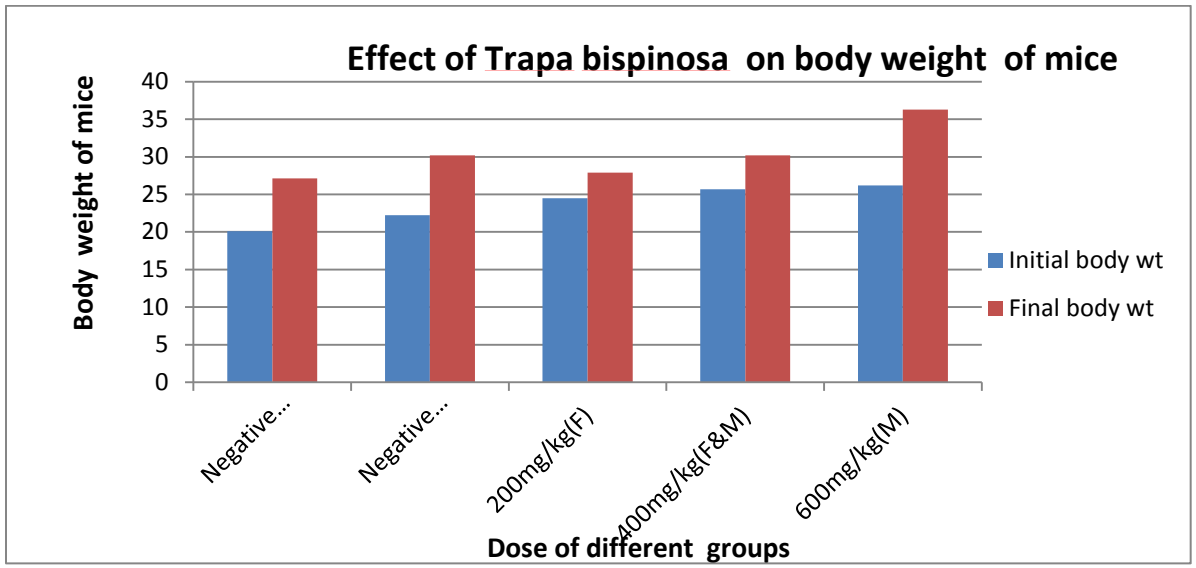


Figure-21: Graphical Presentation of Effect of methanolic extract of *Trapa bispinosa* on body weight in mice

Table-8: Effect of *Trapa bispinosa* 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
Trapa bispinosa	7.96	24.52	69.00	3.72	1.87	0.90

(200mg/kg)						
***Trapa bispinosa* (400mg/kg)**	9.23	29.34	64.20	3.90	4.38	0.88
***Trapa bispinosa* (600mg/kg)**	8.09	28.70	65.00	4.07	1.63	0.60

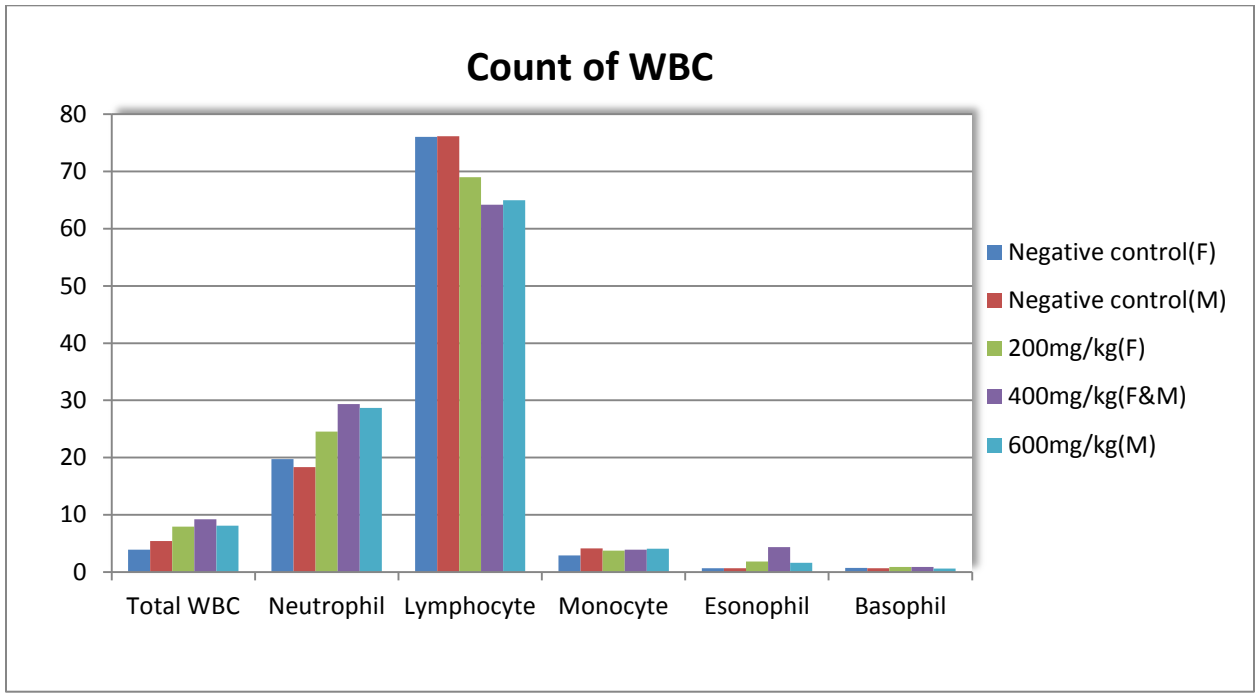


Figure-22 : Effect of *Trapa bispinosa* on the Different count of WBC (White Blood Cell)

Table-9: Effect of *Trapa bispinosa* 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 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
***Trapa bispinosa*(200mg/kg)**	8.70	12.98	40.45	46.70	14.93	32.00	27.08
***Trapa bispinosa*(400mg/kg)**	7.68	12.44	39.82	52.18	16.24	31.26	25.76
***Trapa bispinosa*(600mg/kg)**	8.41	13.33	44.83	53.53	15.93	29.77	24.70

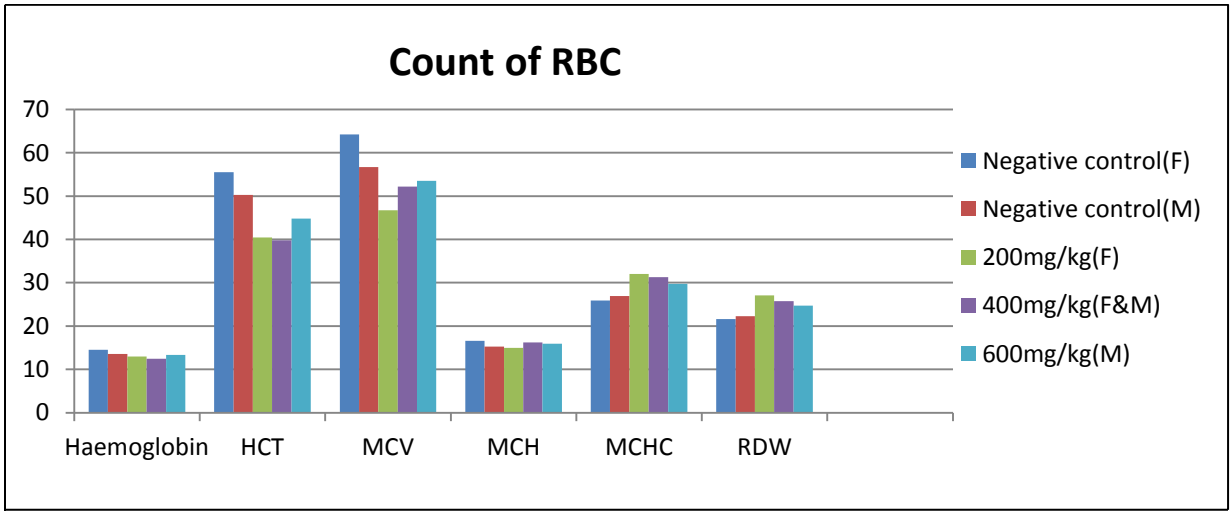


Figure-23: Effect of *Trapa bispinosa* on the count of RBC (Red Blood Cell)

Table-10: Effect of *Trapa bispinosa*on Platelet count on the CBC (Count Blood Cell) Test

Treatment Group	Platelet $10^3/mm^3$(n)
Negative Control group(Female)	848
Negative Control group(Male)	1211
***Trapa bispinosa*(200mg/kg)**	1465.83
***Trapa bispinosa*(400mg/kg)**	990.40
***Trapa bispinosa* (600mg/kg)**	1329.00

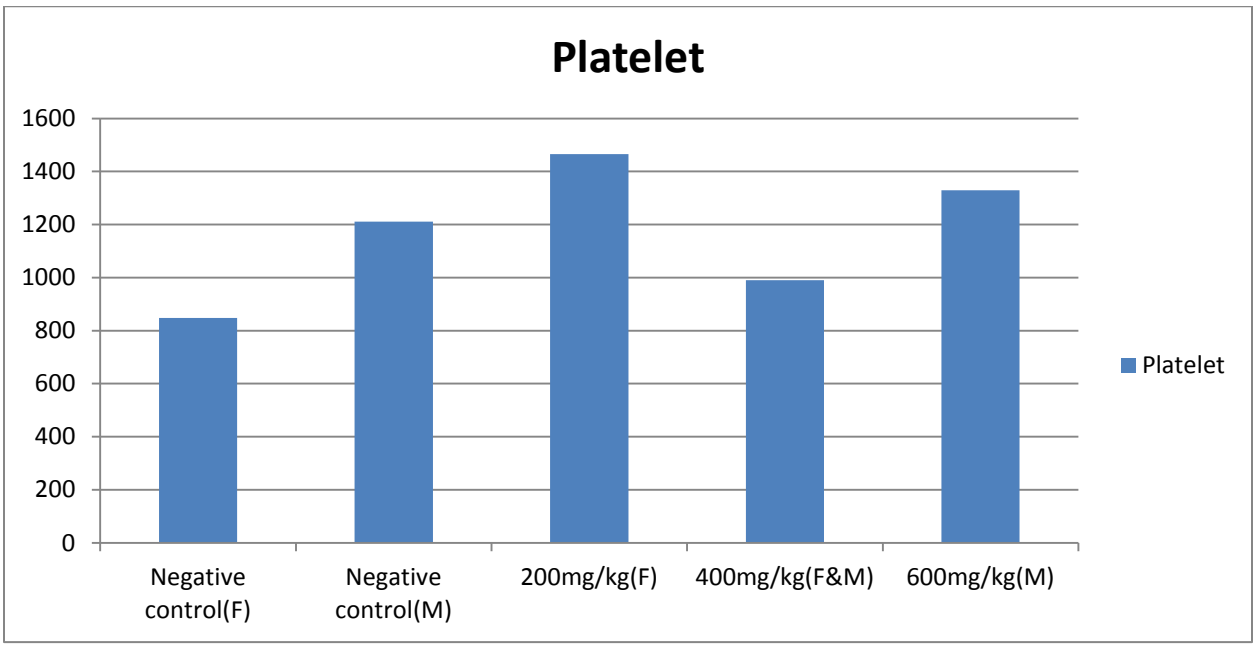


Figure-24: Effect of *Trapa bispinosa* on Platelet on the CBC (Count Blood Cell) Test

Table-11: Effect of *Trapa bispinosa* on the Liver Function Test

Treatment Group	SGPT (IU/dl)	SGOT (IU/dl)	SALP (IU/dl)
Negative Control group(Female)	55.83	33.33	102.17
Negative Control group(Male)	49.17	31.83	341.5
***Trapa bispinosa* (200mg/kg)**	64.25	139.50	152.75
***Trapa bispinosa* (400mg/kg)**	72.00	202.00	137.50
***Trapa bispinosa* (600mg/kg)**	40.50	175.50	140.00

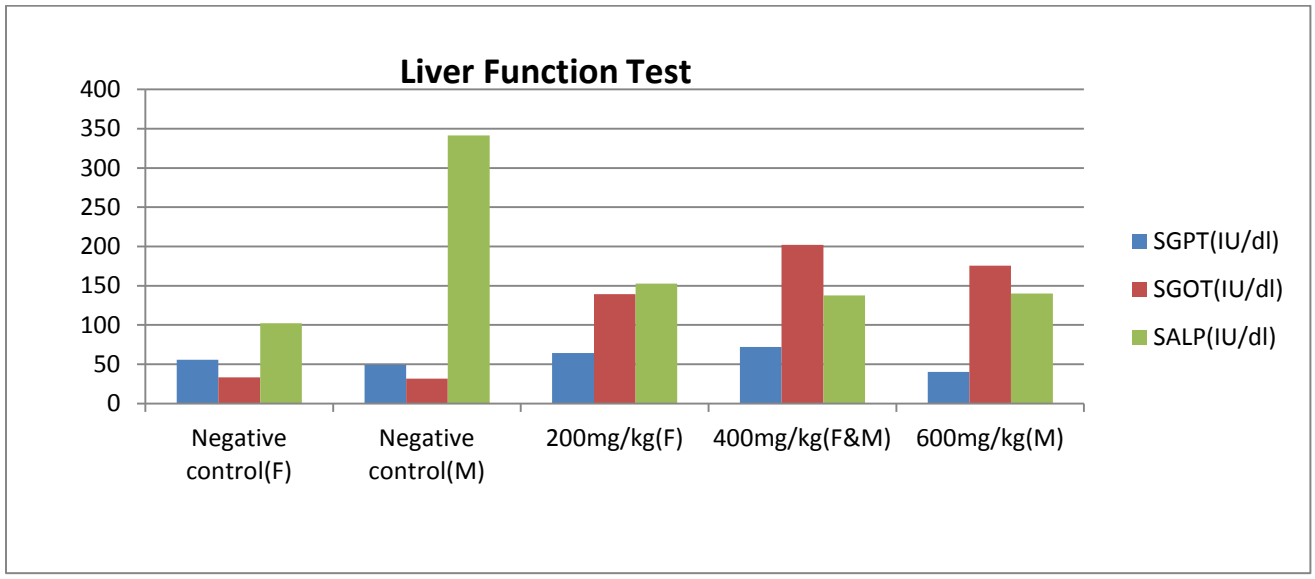


Figure-25: Effect of *Trapa bispinosa* on the Liver Function Test

Chapter- 5
Conclusion

5. 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 Central Nervous System activities, Acute and Chronic toxicity of methanolic extract of *Trapa bispinosa* fruits.

The plant extract was assessed on the central nervous system using a number of neuropharmacological experimental models in mice. The crude extract of *Trapa bispinosa* (200mg/kg, 400mg/kg & 600mg/kg) dose dependently reduces the number of peripheral locomotion, central locomotion and leaning in the open field test. The reduction is significant when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Diazepam 1mg/kg. The crude extract of *Trapa bispinosa* (200mg/kg, 400mg/kg& 600mg/kg) also dose dependently reduces the number of head dipping and head poking in the hole board test. The reduction is significant when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Diazepam 1mg/kg. 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 *Trapa bispinosa* 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 *Trapa bispinosa* 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 *Trapa bispinosa* is safe in mice that is no significant change with dose when compare with negative control.

Chapter- 6
Reference

6.References

Adkar, P., Dongare, A., Ambavade, S. and Bhaskar, V. (2014). Trapa bispinosa Roxb.: A Review on Nutritional and Pharmacological Aspects. *Advances in Pharmacological Sciences*, 2014, pp.1-13.

A. K. Agrahari, M. Khaliquzzama, and S. K. Panda, "Evaluation of analgesic activity of methanolic extract of Trapa natans l.var. Bispinosa roxb. Roots," *Journal of Current Pharmaceutical Research*, vol. 1, pp. 8–11, 2010.

Agarwal T, Shukla DA, Gujrati A, Srivastava N. In vitro analysis of antibacterial activity of *Trapa natans* peel (household waste). IJPRD 2011; 4(suppl 1): 114 – 117.

Alberts, B., (2012), "Table 22-1 Blood Cells", *Molecular Biology of the Cell*, NCBI Bookshelf.

Ambikar DB, Vyawahare NS, Harle UN, Khandare RA, Bore VV. Nootropic activity of hydroalcoholic extract of *Trapa bispinosa* . Pharm Communique (Suppl.) 2008; 45:333-334.

Bhat P., Hegde G.R., Hegde G., Mulgund G.S., (2014).Ethnomedicinal plants to cure skin diseases—An accountof the traditional knowledge in the coastal parts of Central Western Ghats, Karnataka, India. *Journal of Ethnopharmacology*, vol. 151, pp 493–502.

Bitonti MB, Cozza R, Wang G, Ruffini-Castiglione M, Mazzuca S, Castiglione S, SalaF,Innocenti AM, 1996. Nuclear and genomic changes in floating and submerged buds and leaves of heterophyllous waterchestnut (*Trapa natans*). Physiologia Plantarum, 97(1):21-27.

D. Kar, L. Maharana, S. C. Si, M. K. Kar, and D. Sasmal, "Anti-ulcer activity of ethanolic extract of fruit of Trapa bispinosa Roxb. in animals," Der Pharmacia Lettre, vol. 2, no. 2, pp. 190–197, 2010.

D. B. Ambikar, U. N. Harle, R. A. Khandare, V. V. Bore, and N. S. Vyawahare, "Neuroprotective effect of hydroalcoholic extract of dried fruits of Trapa bispinosa roxb on lipofuscinogenesis and fluorescence product in brain of d-galactose induced ageing accelerated mice," Indian Journal of Experimental Biology, vol. 48, no. 4, pp. 378–382, 2010.

Ganong, William, F., (2003), *Review of medical physiology*, (21th eds.), New York: Lange Medical Books/McGraw-Hill, pp. 518.
Ghani, A; (2005). Textbook of Pharmacognosy (Part One); Institute of Medical Technology: Dhaka.

Gupta, B.D., Dandiya, P,C., Gupta, M,L., (1971), "A Psycho-pharmacological Analysis of Behavior in Rat", *Japan J Pharmacol*, vol. 21, pp. 293-298.

Guo JT, Lee HL, Chiang SH, Lin FI, Chang CY. Antioxidant properties of the extracts from different parts of broccoli in Taiwan. J Food Drug Anal 2001; 9: 96-101.

Ghani N. (2002) *Khazainul Advia.* New Delhi: Idara kitabul shifa, pp. 853-854.

Gaillard, Y. and Pepin, G., (1999), Poisoning by plant material: review of human cases and analytical determination of main toxins by high-performance liquid chromatography- (tandem) mass spectrometry, *Journal of. Chromatography*, vol. **733**, pp. 181-229.

Hall, CS. Larsen, HO. Pouliot, M., (2012), "People, plants and health: a conceptual framework for assessing changes in medicinal plant consumption", *Journal of Ethnobiology and Ethnomedicine*, vol. 8, pp. 43.

Hussain, MS. Fareed, S. Ansari, S. Rahman, MA. Ahmad, IZ. Saeed, M. (2012), "Current toward production of plant secendory metabolites", *Journal of Pharmacy and Bioallied Sciences*, approaches vol. 4(1), pp.10- 20.

Hajdu, SI., (1998), The discovery of *Trichomonasvaginalis*, *Act Cytologica*, Vol. 42, pp. 1075.

Hummel M, Kiviat E, 2004. Review of world literature on water chestnut with implications for management in North America. Journal of Aquatic Plant Management, 42:17-28.

Havsteen B. Flavonoids, a class of natural products of high pharmacological potency. Biochem Pharm 1983; 32: 1141-1148.

Howland, RD Publishers. pp. 91., Mary, MJ., (2006), *Lippincott's Illustrated Reviews Pharmacology*.(3rd eds.), Chester: Lippincott Raven -196.

Irikura T, Masuzawa K, Tanaka N, Hasegawa Y, Kawasaki H. Antitumor steroids from *Trapa* fruits. Japanese Kokai Tokkyo Koho 1972; 47(41):522-523.

Jelkmann, W., (2004), Molecular biology of erythropoietin, *Intern Med*, Vol. 43, pp. 649–659.
Johnston, DE., (1999), "Special considerations in interpreting liver function tests", *Am Fam Physician*, Vol. **59** (8), pp. 2223–2230.

Karmakar, U., Rahman, K., Biswas, N., Islam, M., Ahmed, M., Shill, M., Paul, P. and Kamruzzaman, M. (2011). Antidiarrheal, Analgesic and Antioxidant Activities of Trapa bispinosa Roxb. Fruits. *Research Journal of Pharmacy and Technology*, 4(2), pp.294-97.

Kar D, Snigdha P, Maharana L, Dash G.(2004) Hepatoprotective activity ofwater chestnut fruit. Indian J. Nat.Prod, 20, pp. 17-21.

Kar DM, Maharana L, Si S C, Kar MK,Sasmal D. (2010) Anti ulcer activity of ethanolic extract of fruit of Trapabispinosa Roxb. in animals. Der Pharmacia Lettre. 2 (2), pp. 190-197.

Kim B, KimJ, KimH and Heo M. Biological screening of 100 plant extracts for cosmetic use: anti-oxidativeactivity and free radical scavenging activity. International Journal of Cosmetic Science 1997 ;19 (6): 299-307.

Kirtikar KR, Basu BD. Indian medicinal plants. 2nd edn. Vol III. Deharadun: International book distributors; 2006.

Kabeeruddin M. (2010) *Makhzanul Mufradat.* 2nd Ed. New Delhi: Idara kitabul shifa, pp. 269-270.

Loomis, TA. and Hayes, AW., (1996), *Loomis's essentials of toxicology*, (4th eds.), California, Academic press, pp. 208- 245

Maton, A. Jean, H. McLaughlin, C.W. Warner, M.Q. Lattart, D. Wright, J.D., (1993), *Human Biology and Health*, Englewood Cliffs, New Jersey, Prentice Hall.

Mc.Clatchey. Kenneth, D. (2002), *Clinical laboratory medicine*, Lippincott Williams & Wilkins, pp. 288.

Mehrhoff LJ, Silander JA, Leicht SA, Mosher ES, Tabak NM, 2003. *Trapa natans*. IPANE: Invasive Plant Atlas of New England. Storrs, CT: University of Connecticut.

M. A. Razvy, O. F. Mohammad, and A. Mohammad Hoque, "Environment friendly antibacterial activity of water chestnut fruits," Journal of Biodiversity and Environmental Sciences, vol. 1, no. 1, pp. 26–34, 2011.

Makare N, Bodhankar S, Rangari V. Immunomodulato-ry activity of alcoholic extract of *Mangifera indica L* in mice. J Ethnopharmacol 2001; 78: 133-137.

Nyblom H, Berggren U, Balldin J, Olsson R., (2004), "High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking", *Alcohol Alcohol.* Vol. **39** (4), pp. 336–339.
Nyblom H, Berggren U, Balldin J, Olsson R., (2004), "High AST/ALT ratio may indicate advanced alcoholic liver disease rather than heavy drinking", *Alcohol Alcohol.* Vol. **39** (4), pp. 336–339.
Ohl, F. Oitz, MS. Fuchs, E., (1998), "Assessing cognitive functions in tree shrews: visuo-spatial and spatial learning in the home cage", *Journal of Neuroscience Methods*, vol. 81, pp. 35–40.

Philip, C. and Burcham, (2014), *An Introduction to Toxicology,* Target organ toxicity: liver & kidney, Hepatotoxicity, New York: Lange Medical Books/McGraw-Hill, pp. 155.

Poole, A. and Leslie, GB., (1989), *A practical approach to toxicological investigations*, (1st eds.), Great Britain. Cambridge University press, vol. 2, pp. 30-117.

PFAF, 2000. *Trapa natans*. Blagdon Cross, Ashwater, Beaworthy, Devon, UK: Plants for a Future Database.

P. K. Das, S. Bhattacharya, J. N. Pandey, and M. Biswas, "Antidiabetic activity of Trapa natans fruit peel extract against streptozotocin induced diabetic rats," Global Journal of Pharmacology, vol. 5, no. 3, pp. 186–190, 2010.

Patel SA, Patel NC, Shah MH, Shah VN. Evaluation of anti-inflametory activity of fruit of *Trapa natans.* International Journal of Pharmaceutical Research and Development 2011; 3:97-102.

Paul, N. Roy, R. Bhattacharya, S. Biswas, M., (2012), "Acute and sub-chronic toxicity study of *Cocos nucifera* leaf extracts in mice", *Journal of Advanced Pharmacy Education & Research* , vol. 2 (2), pp. 74-81.

Perez G.R.M., Perez I.J.A., Garcia D., Sossa M.H., (1998). Neuropharmacological activity of Solanum nigrum fruit. *J. Ethnopharmacol*., vol. 62, pp.43-48.

Prajapati ND, Purohit SS, Sharma AK, Kumar T. A hand book of medicial plant .1st ed . Agrobios (india) publishe, jodhpur 2003;519-520.

Prajapati N D, Kumar U. (2005) *Agro's Dictionary of Medicinal plants.* Jodhpur: Agrbios, pp. 351.

Pratt, DS., (2010), Liver chemistry and function tests, Feldman, M. Friedman, LS. Brandt, LJ., (9^{th} eds), *Sleisenger and Fordtran's Gastrointestinal and Liver Disease*, Philadelphia, Pa: Saunders Elsevier; chap 73.

Purves, William, K. David, S. Gordon, H. Orians, H. Craig, H., (2004), *Life: The Science of Biology*, (7th eds.), Sunderland, Mass: Sinauer Associates, pp. 954.

Rahman MM, Mosaddik A, Wahed MII, Haque ME. Antimicrobial activity and cytotoxicity of Trapa bispinosa. Fitoterapia 2000,71:704-706.

Rahman M.M., Raysal M.J., Alam M., (2007). Medicinal plants used by Chakma tribe in Hill Tracts districts of Bangladesh, Indian. *Journal of Traditional Knowledge*, vol. 6, pp. 508-517.

Ramsay, G., (1998), *Commercial biosensors*, John Wiley & Sons, Inc, New York.

Robert, BT. Frederic, M. Timmons, Michael, J., (2006), *Human anatomy*, (5th eds.), San Francisco: Pearson/Benjamin Cummings, Pp. 529.

Skibola, CF. Smith, M.T., (2000), Potential health impacts of excessive flavonoid intake, *Free radical biology & medicine*, vol. **29**, pp. 375-383.

Song MC, Yang HJ, Lee D Y, Ahn EM, Kim DK, Kim JY, Chung DK, Baek N I :Triterpenoids from Trapa pseudoincisa. J. Appl. Biol. Chem. 2007;50(4): 259-263.

Timbrell, J., (2002), *Introduction to toxicology*, (3rd eds.), London, Taylor & Francis, pp. 163-179.
Tomlinson, TR. Akerele, O., (1998), *Medicinal plants their role in health and biodiversity*, Philadelphia, University of Pennsylvania press, pp. 29-40.

Tonnesen, H. Hejberg, L. Frobenius, S. Andersen, J., (1986), "Erythrocyte mean cell volume--correlation to drinking pattern in heavy alcoholics", *Acta Med Scand*, Vol. **219** (5), pp. 515–518.
Uddin and Hassain (2010). Angiosperm diversity of Lawachara National park (Bangladesh): A preliminary. Bangladesh Association of Plant Taxonomists, vol. 17(1), pp. 9-22.

Varalakshmi, KN. Sangeetha, CG. Samee, US. Irum, G. Lakshmi, H. Prachi, SP., (2011), "In Vitro Safety Assessment of the Effect of Five Medicinal Plants on Human Peripheral Lymphocytes", *Tropical Journal of Pharmaceutical Research*, vol. 10 (1), pp. 33-40.

Wang H, Cao G, Prior RL. Total antioxidant capacity of fruits. J Agric Food Chem1996; 44: 701-705.

Wahed TB, Islam MF, Sarkar R and Wahed SB: Phytochemical, antioxidant and cytotoxicity study of the methanolic extracts of leaves of *Trapa bispinosa* Roxb.Int J Pharm Sci Res2014; 5(10): 4400-05.doi: 10.13040/ IJPSR.0975-8232.5(10).4400-05.

Williamson, EM. Okpado, DT. Evans, FJ., (1996), *Selection, preparation and pharmacological evaluation of plant material. England*, John Wiley& sons, pp. 1-25.

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Annexure

List of Abbreviation	Full Meaning
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ALP	Alkaline Phosphatase
ANOVA	One-way Analysis of Variance
CBC	Complete Blood Count
CMC	Carboxy Methyl Cellulose
CNS	Central Nervous System
HCT	Hematocrit
ICDDR, B	International Centre for Diarrhoeal Disease and Research, Bangladesh
ITIS	Integrated Taxonomic Information System
LFTs or LFs	Liver Function Tests
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Cell Volume
NCCAM	National Center for Complementary & Alternative Medicine
PNS	Peripheral Nervous System
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