Pharmacological Studies of Methanolic Extract of *Glycosmis pentaphylla*

This Thesis Paper is submitted in Partial Fulfillment of the Requirement for the Degree of Bachelors of Pharmacy, East West University

Submitted by

Tahiya Islam

ID: 2013-1-70-002

Supervised by

Dr. Shamsun Nahar Khan

Ph. D, Postdoc, Harvard University

Chairperson and Associate Professor

Department of Pharmacy

East West University

Submission Date: 19 December, 2016

This Research paper is dedicated to my beloved Parents

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled "**Pharmacological studies of Methanolic Extract of *Glycosmis pentaphylla***" is an authentic and genuine research work carried out by me under the guidance of Dr. Shamsun Nahar Khan, Chairperson and Associate Professor, Department of Pharmacy, East West University, Dhaka.

_____

Tahiya Islam

ID # 2013-1-70-002

Department of Pharmacy

East West University, Dhaka.

ENDORSEMENT BY HEAD OF THE DEPARTMENT

This is to certify that the dissertation entitled "**Pharmacological studies of Methanolic Extract of *Glycosmis pentaphylla***" is a genuine research work carried out by Tahiya Islam, under the supervision of Shamsun Nahar Khan (Ph. D, Postdoc, Harvard University, Chairperson, Department of Pharmacy, 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 thus connection are duly acknowledged.

Dr. Shamsun Nahar Khan
Ph. D, Postdoc, Harvard University
Chairperson and Associate Professor
Department of Pharmacy
East West University

CERTIFICATE

This is to certify that, the thesis on "**Pharmacological studies of Methanolic Extract of *Glycosmis pentaphylla*"** submitted to Department of Pharmacy, East West University, Aftabnagar, Dhaka, in partial fulfillment of the requirements for the degree of Bachelors of Pharmacy (B. Pharm), was carried out by Tahiya Islam (ID # 2013-1-70-002) under my guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information of in this connection are duly acknowledged.

_____

Dr. Shamsun Nahar Khan
Ph. D, Postdoc, Harvard University
Chairperson and Associate Professor
Department of Pharmacy
East West University
Aftabnagar, Dhaka

ACKNOWLEDGEMENT

Above all, I express my gratitude to Almighty Allah for giving me the strength, energy and patience to carry out this research work. I would like to express my gratitude and admiration to **Dr. Shamsun Nahar Khan**, Associate Professor and Chairperson, Department of Pharmacy, East West University, for her suggestion, careful guidance, sincere help, constructive criticism and valuable time without which I would not have been able to complete this work. She had been very enthusiastic and supportive in my research.

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

I would also like to take the opportunity to express my whole hearted gratitude to my fellow researcher friends specially Nadia Akther, Mohammad Ali and near and dear ones who offered encouragement, information, inspiration and assistance during the period of constructing the research report .

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

ABSTRACT

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

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 the 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 *Glycosmis pentaphylla* (200mg/kg, 400mg/kg and 800mg/kg) dose dependently reduces the number of peripheral locomotion, central locomotion and leaning in the open field test and reduces the number of head dipping and head poking in the hole board test. The reduction is significant when it is compared to the standard drug.

The aim of the study was also to investigate the possible toxicity of the plant *Glycosmis pentaphylla* and especially to establish the safety of the methanolic extract of this plant by focusing on its chronic toxicity in mice. For finding chronic toxicity several tests were done such as CBC (Cell Blood count) test and Liver Function test.

All data were analyzed by using SPSS analytical method.

Conclusion: After summarizing all the results, it can be said that *Glycosmis pentaphylla* may have several pharmacological activities but to prove the hypothesis, further higher studies are needed to be conducted.

Keywords: *Glycosmis pentaphylla,* Neuro pharmacological effect and Toxicity test.

Table of Contents

Chapter 1	**Introduction**	**1-35**
1.1	Medicinal Plants	1
1.1.1	Medicinal plants all around the world	1
1.1.2	History of medicinal plants	2-3
1.1.3	Traditional and alternative medicine	3-4
1.1.4	Future of medicinal plants	4
1.1.5	Characteristics of medicinal plants	4
1.1.6	Medicinal plants in Bangladesh	5
1.1.7	Practice of traditional medicine in Bangladesh	5
1.2	Herbal products and their toxicity profiles	6
1.3	Nervous system	6-7
1.3.1	Central nervous system	7-8

1.3.2	Peripheral nervous system	8-9
1.3.3	Somatic nervous system	9
1.3.4	Autonomic nervous system	9
1.3.5	Nerve cells	10
1.3.6	Cell body or soma	10
1.3.7	Axon	10
1.3.8	Dendrite	11
1.3.9	Synapse	11
1.3.10	Different central nervous system disorders	11-12
1.4	Toxicology	12
1.4.1	Toxicity	13
1.4.2	Exposure	13
1.4.3	Route of exposure	13-14
1.4.4	Acute toxicity	14
1.4.5	Chronic toxicity	14
1.4.6	Evaluation of herbal toxicity	14-15
1.5	Hematology	15

1.5.1	Cellular elements of blood	15-16
1.5.2	Plasma	16
1.5.3	Red Blood Cell	16-17
1.5.4	Count of Red Blood Cell	17-25
1.5.5	White Blood Cell	25-26
1.5.6	Platelets	27-28
1.5.7	Hepatotoxicity	28-29
1.5.8	Liver	29-31
1.5.9	Liver Function tests	31-35
Chapter 2	**Plant Introduction**	**36**
2.1	Introduction of the Plant	37
2.1.1	Description of *Glycosmis pentaphylla*	37-38
2.2	Description	38-39
2.3	Geographical distribution	39
2.4	Chemical composition	39-41
2.5	Medicinal uses	41

Chapter 3	**Materials and methods**	**42**
3.1	Preparation of plant extraction	43-44
3.1.1	Crystal formation	44
3.2	Experimental Animals	44-45
3.3	CNS Activity Test	45
3.3.1	Materials for CNS Activity Test	45
3.3.2	Chemical Agents Used in Test	45
3.3.3	Doses Used in CNS Activity Test of the Extract:	45-46
3.3.4	Methods for CNS Activity Test:	46
3.3.5.1	Open Field Test:	46-47
3.3.5.2	Hole Board Test	47-48
3.4	Toxicity Test	48
3.4.1	Materials for Toxicity Test	48

3.4.2	Chemical Agents Used Toxicity Test	49
3.4.3	Doses Used for Toxicological Activity of the Extract	49
3.5	Chronic Toxicity Test	49
3.6	Hematological parameters	49
3.7	Serum biochemical parameters	49
3.8	Histopathological studies	49
3.9	Statistical Analysis	50
Chapter 4	**Results and Discussion**	**51**
4.1	Consequences of dosing:	52
4.2	CNS Activity Test of Methanolic Extract of *Glycosmis pentaphylla*	53
4.2.1	Open Field Test	53
4.2.1.1	Total Number of Peripheral locomotion, Central locomotion, Leaning, Rearing	53- 57
4.2.1.4	Hole Board Test:	58-59
4.3	Chronic Toxicity Test:	60-67
	Conclusion	68-69
Chapter 5	**Reference**	**70-73**

List of Figure

Serial	Title	Page
Figure 1	Classification of Nervous system	7
Figure 2	Different parts of brain	8
Figure 3	Peripheral Nervous System	9
Figure 4	Neuron	10
Figure 5	Cellular elements of blood	15
Figure 6	Blood plasma	16
Figure 7	Red Blood Cells	17
Figure 8	Structure of hemoglobin	18
Figure 9	Hematocrit Determination	20
Figure 10	Equation to determine the percent of hematocrit	22
Figure 11	Equation to determine MCV, MCH and MCHC	24
Figure 12	White Blood Cell	25
Figure 13	Various kinds of White Blood Cells	26

Figure 14	Platelet	27
Figure 15	Platelet aggregation to form a blood clot	28
Figure 16	Human Liver	30
Figure 17	Mouse Liver	31
Figure 18	3D Structure of Alkaline Phosphatase	33
Figure 19	3D structure of Aspartate Transaminase	34
Figure 20	*Glycosmis pentaphylla*	39
Figure 21	Herbarium Sheet of *Glycosmis pentaphylla*	43
Figure 22	Rotary filter for crystal formation	44
Figure 23	Swiss albino mice	45
Figure 24	Open field test	47
Figure 25	Hole board test	48
Figure 26	Consequences of dosing	52
Figure 27	Graphical representation of number of central locomotion	54
Figure 28	Graphical representation of number of peripheral locomotion	55
Figure 29	Graphical representation of number of leaning	56
Figure 30	Graphical representation of number of head poking and dipping	58
Figure 31	Sacrifice of a Swiss albino mice	59
Figure 32	Graphical representation of different counts of WBC	61
Figure 33	Graphical representation of different counts of RBC	62
Figure 34	Graphical representation of platelet count	63
Figure 35	Graphical representation of Liver Function test	64
Figure 36	Graphical representation of the initial and final body weight of female mice.	65
Figure 37	The damaged and enlarged spleen of GP4F3 mice	66

Figure 38	The attached and enlarged spleen and liver of GP8F8	67
Figure 39	Sacrifice of a female mice where the internal organs are attached to one another	68

List of Table

Serial	Title	Page
Table 2.1	Chemical composition of *Glycosmis pentaplylla*	40-41
Table 4.2.1.2	CNS Activity of plant extract of *Glycosmis pentaplylla* by Open Field Test (Central Locomotion) in Mice.	53
Table:4.2.1.3	CNS Activity of plant extract of *Glycosmis pentaplylla* by Open Field Test (Peripheral Locomotion) in Mice.	55
Table 4.2.1.4	CNS Activity of plant extract of *Glycosmis pentaplylla* by Open Field Test (Leaning) in Mice.	56
Table 4.2.1.5	CNS Activity Test of Methanolic Extract of *Glycosmis pentaplylla* by hole board test in mice.	58
Table 4.5	Effect of *Glycosmis pentaplylla* on the different count of WBC(White Blood Cell)	62
Table 4.6	Effect of *Glycosmis pentaplylla* on the Different count of RBC	63
Table 4.7	Effect of *Glycosmis pentaplylla* on Platelet count on the CBC	64
Table 4.8	Effect of *Glycosmis pentaplylla* on the Liver Function Test	65
Table 4.9	Effect of *Glycosmis pentaphylla* on the ody weight of the female mice	66

Annexure

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

PNS	Peripheral Nervous System
PT	Prothrombin Time
PV	Polycythemia Vera
RBC	Red Blood Cell
RDW or RCDW	Red Blood Cell Distribution Width
RPM	Rotation Per Minute
SALP	Serum Alkaline Phosphatase
SEM	Standard Error Mean
SGOT	Serum Glutamate Oxaloacetate Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
SPSS	Statistical Package for the Social Science
WBC	White Blood Cell

Chapter 1
Introduction

1.1 Medicinal plants:

The plants which have therapeutic and biological activities are known as the medicinal plants. These plants are of great importance to mankind. They are not only used in the treatment of various diseases but also are used in various creative and cultural purposes all around the world by the human race. Medicinal plants also have impacts on drug design and drug development and different drug synthesis. Many compounds are made from these plants which act as defenses against fungi, bacteria, viruses, insects and herbivorous mammals. Chemical compounds in these plants mediate their effect on the human body through processes exactly like the conventional drugs do. So, in terms of mechanism of action, these plants do not significantly differ from the conventional drugs. This enables herbal medicines to provide beneficial pharmacological activities as well as harmful side effects. (Wikipedia)

1.1.1 Medicinal plants all around the world:

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

There are a huge number of medicinal plants. In the US, almost 1800 medicinal plant species are commercially available. It has been estimated that about 13,000 species of plants have been employed for at least a century as traditional medicines by various cultures around the world. A list of over 20,000 medicinal plants has been published, and very likely a much larger number of the world's flowering plant species have been used medicinally. Sometimes the figure of 70,000 medicinal plant species is cited, but this includes many algae, fungi, and micro-organisms that are not really plants as the word is understood by botanists. In any event, there is no other category of plants useful to man (with the possible exception of ornamental plants) that includes so many species, and the question naturally arises why such a staggering number of plants have useful medicinal properties. (Ghani, 2003)

1.1.2 History of medicinal plants:

- According to World Health Organization (WHO), about 70 percent of the world's population relies on plants for their primary health care and some 35,000 to 70,000 species has been used as medicaments, a figure corresponding to 14-28% of the 250,000 plants species estimated to occur around the world, and equivalent to 35-70% of all species used world-wide.
- In today's global market, more than 50 major drugs originated from tropical plants. From about 250,000 species of higher plants around the world, only 17% have been scholarly investigated for medical potential.
- The chemical and biological diversity of plants represent a potentially limitless renewable source for the use in the development of new pharmaceuticals. Flora of China and North America have almost the same numbers of flowering plants around 35,000. However, traditional Chinese medicine use 5000 of them, but Native Americans used 2564 medicinal plants. North American herbal medicine represents a rich, yet unexplored source of potential phyto-pharmaceuticals.
- According to American ethnobotanist Daniel Moerman, Native Americans used about 9% of all vascular flora for medicinal purposes. Yet, only a few screenings of North American medicinal plants have ever been undertaken, and vast majority of plants still remain unknown.
- The botanical wisdom accumulated by indigenous people has led to the establishment of the traditional systems of medicine including Chinese, Ayurvedic, Middle Eastern, European, African and American.
- According to American pharmacognosist Norman Farnsworth, 89 plant-derived drugs currently prescribed in the industrial world were discovered by studying traditional herbal use, an ethnobotanical approach.
- In 18th century, British doctor William Withering discovered effectiveness of foxglove (Digitalis purpurea) from traditional European herbal medicine, for treatment of dropsy. The retention of fluid also was alleviated by the administration of foxglove.
- In 20th century, more than 30 cardiac glycosides have been isolated from dried foxglove leaves including digitoxin and digoxin. Cardiac glycosides are useful

Because they increase the force of cardiac contractions, and allow the heart with more time to rest between contractions. Each year, over 1500 kilograms of digoxin and 200 kilograms of digitoxin were prescribed to heart patients throughout the world.

- Rauvolfia serpentine, the snakeroot plant, traditionally is used for treatment of insomnia in Ayurvedic medicine of India. In 1949, German chemists extracted alkaloid reserpine from Rauvolfia, roots used today for high blood pressure treatment.
- Currently, major biologically active compound in fighting malaria is artemisinin, a sesquiterpene lactone, first isolated in 1972 from wormwood (Artemisia annua) by Chinese chemists studying traditional Chinese herbal medicine.
- Research on traditional medicinal plants in the U.S. has resulted in the discovery of alkaloids from Madagascar periwinkle (Catharanthusroseus), used in the chemotherapy of childhood leukemia and for treatment of Hodgkin's disease. The compound taxol with anti-cancer action was discovered in the bark of Pacific yew tree (Taxusbrevifolia), and approved by FDA in 1992. Understanding the relationship among medicinal plants used in traditional medicine systems can help identify plant materials with potential constituents applicable to modern medicine.
- Studies indicate that the traditional medicine of Native Americans used plants from the same family and genus, as the Chinese used in their traditional medicine system. For example, Asian ginseng (Panax ginseng) and American ginseng (Panaxquinquefolius) were used as adaptogenic plants; similarly in Chinese and Native American traditional medicine. American licorice (Glycyrrhizalepidota) and Asian licorice (Glycyrrhizaglabra) were used in the same way for treatment of bronchial asthma in traditional medicine of China and North America. (Mamedov, 2012)

1.1.3 Traditional and alternative medicine:

The term **"Traditional medicine"** is the sum total of knowledge, skill and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.

The term **"alternative medicine"** refer to a broad set of health care practices that are not part of that country's own tradition or conventional medicine and are not fully integrated into the

dominant health-care system. They are used interchangeably with traditional medicine in some countries. (World Health Organization)

These days, the term **"Alternative Medicine"** became very common in western culture, it focuses on the idea of using the plants for medicinal purpose. But the current belief is that medicines which come in capsules or pills are the only medicines that we can trust and use. Even so most of these pills and capsules we take and use during our daily life came from plants.

Medicinal plants are frequently used as raw materials for extraction of active ingredients which used in the synthesis of different drugs. Like in case of laxatives, blood thinners, antibiotics and anti-malaria medications, contain ingredients from plants. Moreover the active ingredients of Taxol, vincristine, and morphine isolated from foxglove, periwinkle, yew, and opium poppy, respectively. (Hassan, 2012)

1.1.4 Future of Medicinal Plants:

Medicinal plants have a promising future because there are about half million plants around the world, and most of them their medical activities have not investigate yet, and their medical activities could be decisive in the treatment of present or future studies.

1.1.5 Characteristics of Medicinal Plants:

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

Synergic medicine- The ingredients of plants all interact simultaneously, so their uses can complement or damage others or neutralize their possible negative effects.

Support of official medicine- In the treatment of complex cases like cancer diseases the components of the plants proved to be very effective.

Preventive medicine- It has been proven that the component of the plants are also characterized by their ability to prevent the appearance of some diseases. This will help to reduce the use of the chemical remedies which will be used when the disease is already present i.e., reduce the side effects of synthetic treatment. (Hassan, 2012)

1.1.6 Medicinal plants in Bangladesh:

It has been recorded that about 450 or 500 plants that grow or are available in Bangladesh have therapeutic values. In Bangladesh, people who live in the remote hilly areas, like the ethnic communities mostly reply on the use of herbal medicines. As the herbal medicines have huge potentials, there are twenty pharmaceutical companies which manufacture herbal medicines in our country. About six thousand metric tons of medicinal plants are required annually by the relevant industries in Bangladesh for producing traditional medicines. (Motaleb, 2011)

1.1.7 Practice of traditional medicine in Bangladesh:

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

1.2 Herbal products and their toxicity profiles:

Many plant products are toxic. In a herbal supplement, one product may produce the desired therapeutic effect and the other ingredients may produce toxicity, since all the compounds are

extracted together in making herbal supplements and good products are not usually separated from bad products, toxicity may occur from common herbal supplements.

Some herbal supplements may produce toxicity because of the size of the dose. .From the past few decades, plants make an important contribution to healthcare. Herbal preparations contain complex mixtures of one or more plants which contain active ingredients, plant materials in crude or processed form. The data existing for most plants to assurance their quality, efficacy and safety is unacceptable. Majority of people who use herbal medicines do not inform their physician about their use. Herbal medicines can alter physiology and these changes can be reflected in irregular test results.

There are some scientific evidences, which prove that the herbal medicines can cause considerable toxicities. So proper control of the herbal supplements is needed. For example-

- Kava is a herbal sedative anti-anxiety or calming effect, and to relieve symptoms of throat pain as it produces a ―numbing effect on the tongue and throat.
- Toxicity Kava can have additive effects with central nervous system depressants.
- A patient who was taking alprazolam, cimetidine and terazosin became lethargic and disoriented after ingesting kava.
- Kava lactones can inhibit cytochrome P-450 activities and have a potential interaction with the drugs that are metabolized by the liver.
- Heavy consumption of kava has been associated with increased- gultamyltranferase. (Srivalli et al,2011)

1.3 Nervous system:

The nervous system has three main functions: sensory input, integration of data and motor output. Sensory input is when the body gathers information or data, by way of neurons, glia and synapses. The nervous system is composed of excitable nerve cells (neurons) and synapses that form between the neurons and connect them to centers throughout the body or to other neurons. These neurons operate on excitation or inhibition, and although nerve cells can vary in size and location, their communication with one another determines their function.

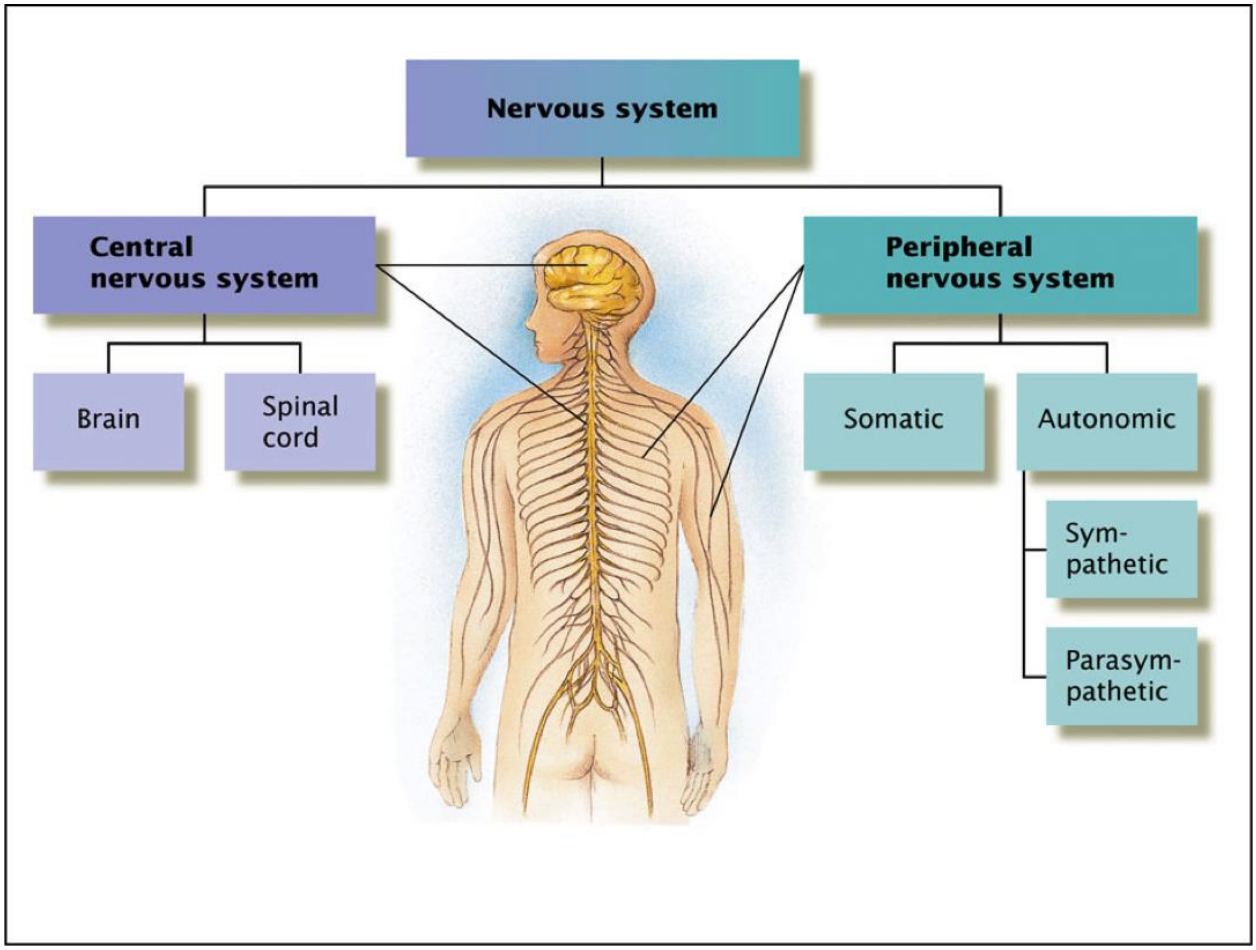


Figure 1: Classification of Nervous System (Keating, 2015)

1.3.1Central 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)

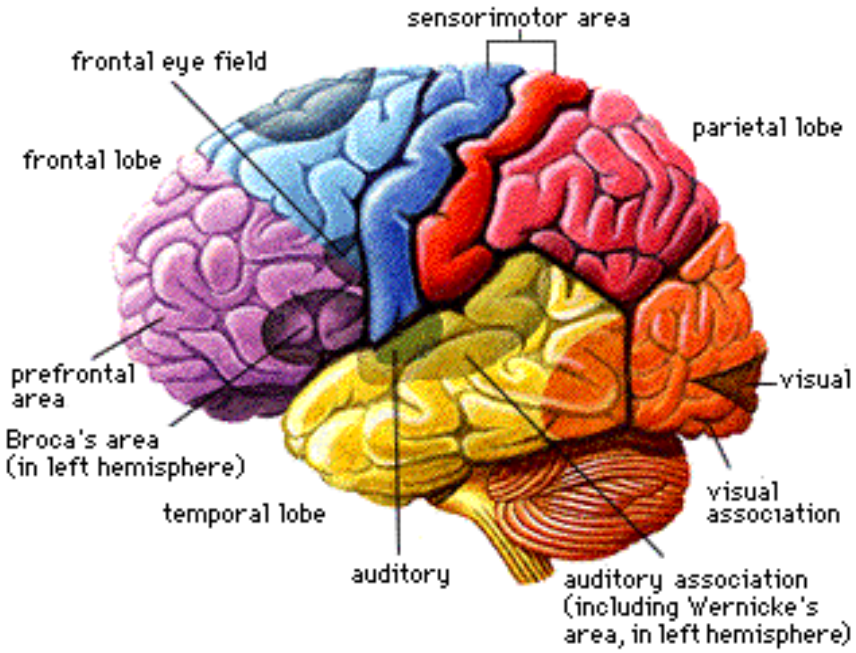


Figure 2: Different parts of brain

The CNS consists of-

- Brain and spinal cord
- Medulla
- Pons
- Cerebrum

1.3.2 Peripheral nervous system:

The PNS is a vast network of spinal and cranial nerves that are linked to the brain and the spinal cord. It contains sensory receptors which help in processing changes in the internal and external environment. This information is sent to the CNS via afferent sensory nerves. The PNS is then subdivided into the autonomic nervous system and the somatic nervous system.

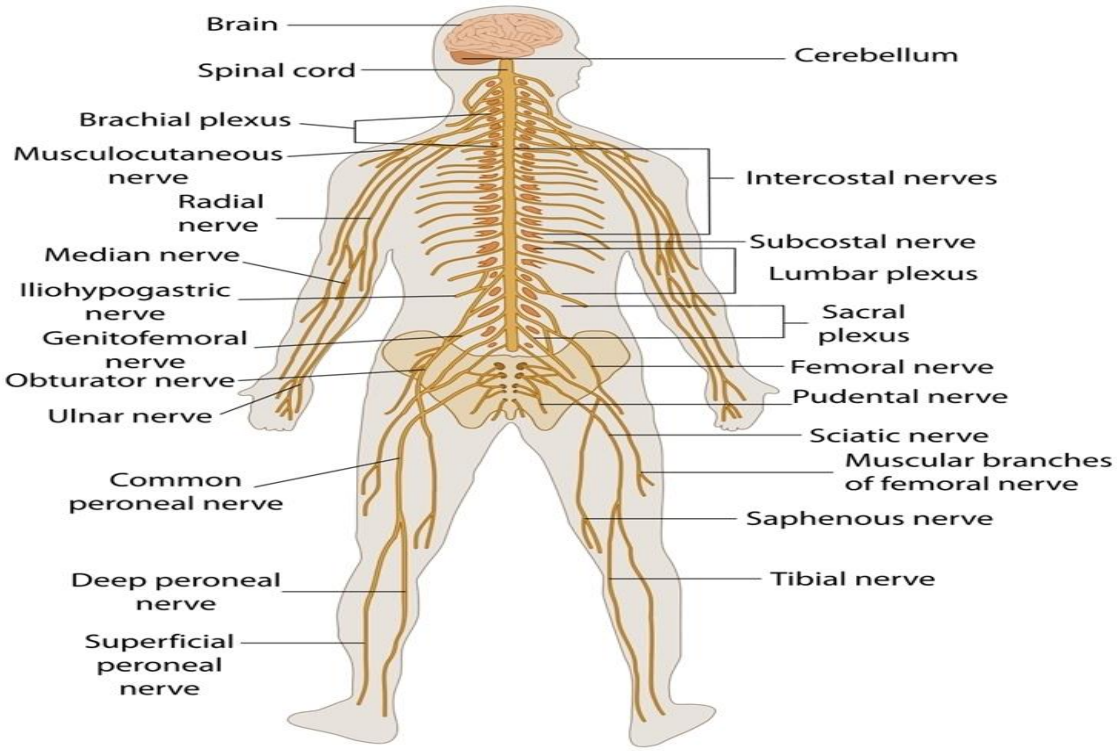


Figure 3: Peripheral Nervous System

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

1.3.3 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.

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

1.3.5 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.

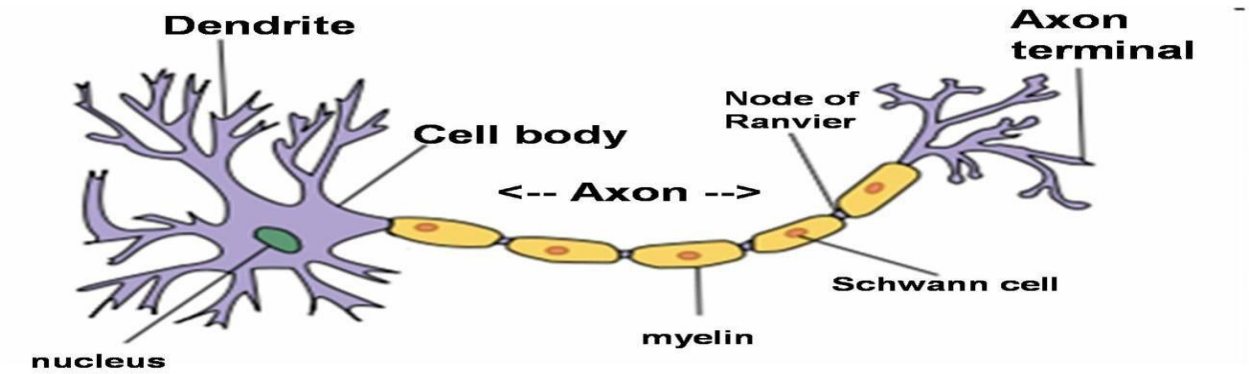


Figure 4: Neurons or Nerve cells

1.3.6 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.

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

1.3.8 Dendrites:

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

1.3.9 Synapse

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

1.3.10 Different central nervous system disorders

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

- ✓ **Guillain-Barré syndrome**- A disorder in which the body's immune system attacks part of the nervous system.
- ✓ **Headache (primary)**-Includes tension (muscular contraction), vascular (migraine), and cluster headaches not caused by other underlying medical conditions.
- ✓ **Headache (secondary)-**Includes headaches that result from other medical conditions.

  These may also be referred to as traction headaches or inflammatory headaches.
- ✓ **Meningitis**-An inflammation of the meninges, the membranes that cover the brain
- ✓ **Multiple sclerosis (MS)**-A disease of the central nervous system that is an unpredictable condition that can be relatively benign, disabling, or devastating, leaving the patient unable to speak, walk, or write.
- ✓ **Parkinson's disease (PD)**-The most common form of parkinsonism; a slowly progressing, degenerative disease that is usually associated with the following symptoms, all of which result from the loss of dopamine-producing brain cells: tremor or trembling of the arms, jaw, legs, and face; stiffness or rigidity of the limbs and trunk; bradykinesia (slowness of movement); postural instability, or impaired balance and coordination.
- ✓ **Seizure**- Occurs when part(s) of the brain receives a burst of abnormal electrical signals that temporarily interrupts normal electrical brain function. **(**Howland and Mycek, 2006).

1.4 Toxicology:

The traditional definition of toxicology is "the science of poisons." As our understanding of how various agents can cause harm to humans and other organisms, a more descriptive definition of toxicology is "the study of the adverse effects of chemicals or physical agents on living organisms". Toxic substances may be systemic toxins or organ toxins. A systemic toxin is one that affects the entire body or many organs rather than a specific site. For example, potassium cyanide is a systemic toxicant in that it affects virtually every cell and organ in the body by interfering with the cell's ability to utilize oxygen. Toxicants may also affect only specific tissues or organs while not producing damage to the body as a whole. These specific sites are known as the target organs or target tissues. Some examples: Benzene is a specific organ toxin in that it is primarily toxic to the blood-forming tissues. Lead is also a specific

organ toxin; however, it has three target organs (central nervous system, kidney, and hematopoietic system).

1.4.1 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.

1.4.2 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.

1.4.3 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)

1.4.4 Acute toxicity:

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

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

1.4.5 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 nonlethal dose (as established in rodent acute toxicity studies) (Poole and Leslie, 1989; Loomis and Hayes, 1996)

1.4.6 Evaluation of herbal toxicity:

(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) Exposed cells, sub-cellular fractions or single-celled organisms to the plant material *(in vitro)* (Timbrell, 2002).

1.5 Hematology

Hematology deals 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. 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).

1.5.1 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)

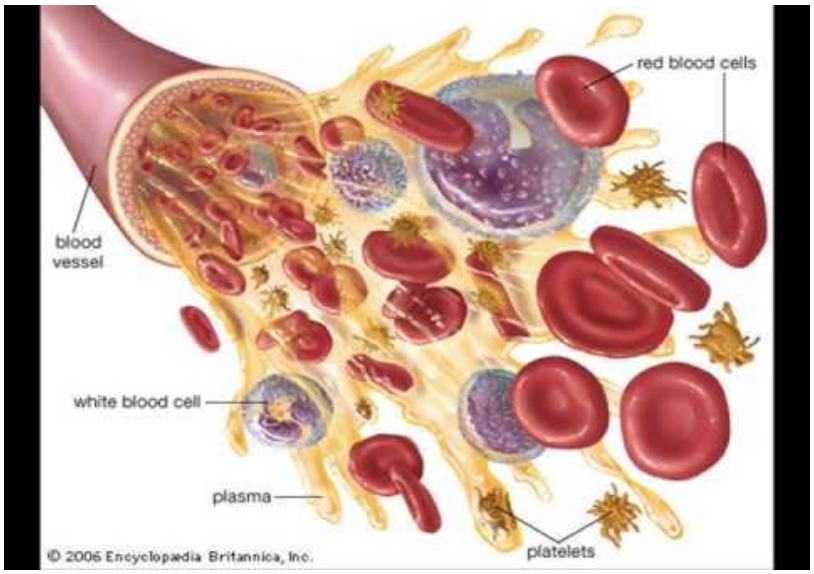


Figure 5: Cellular elements of blood

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).

1.5.2 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), waste of metabolic exchange (urea, ammonia), hormones, and vitamins.

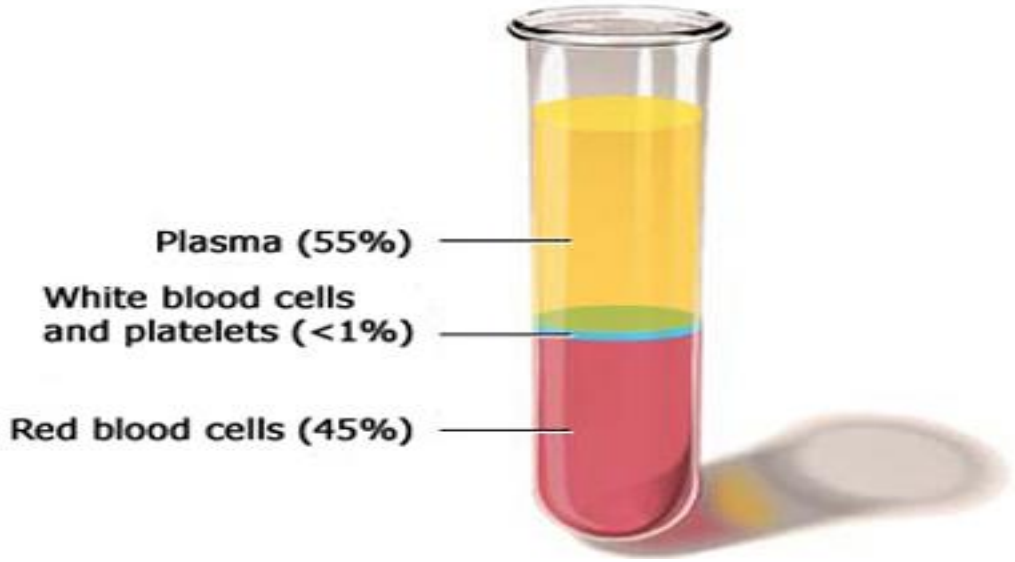


Figure 6: Blood Plasma

1.5.3 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, human body 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. It 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 8-16×106mm3(Robert, et.al. 2006).

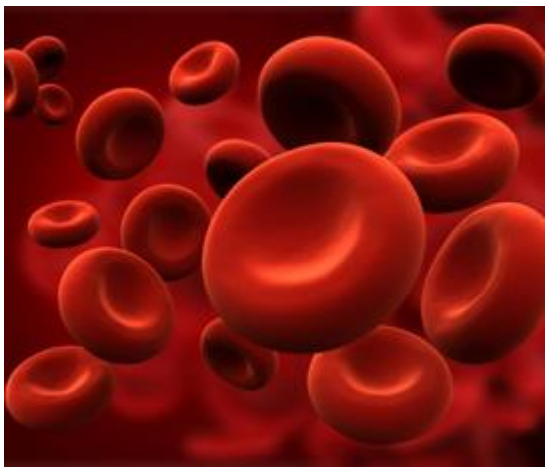


Figure 7: Red blood cell

1.5.4 Count of RBC:

i. **Hemoglobin:** Hemoglobin is the iron-containing oxygen-transport metallo-protein 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.

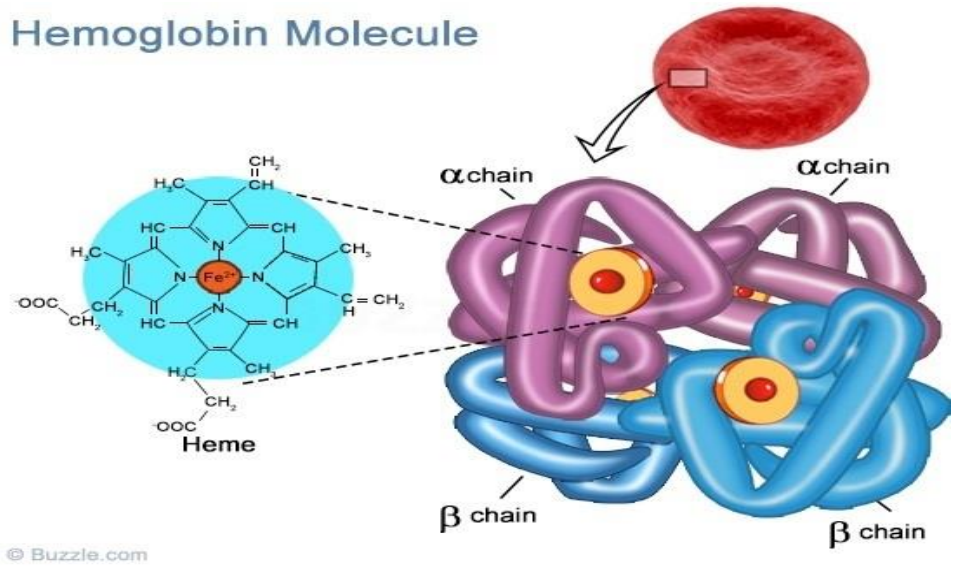


Figure 8: Structure of Hemoglobin molecule

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 tractor 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 (corpulmonale)
- 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).

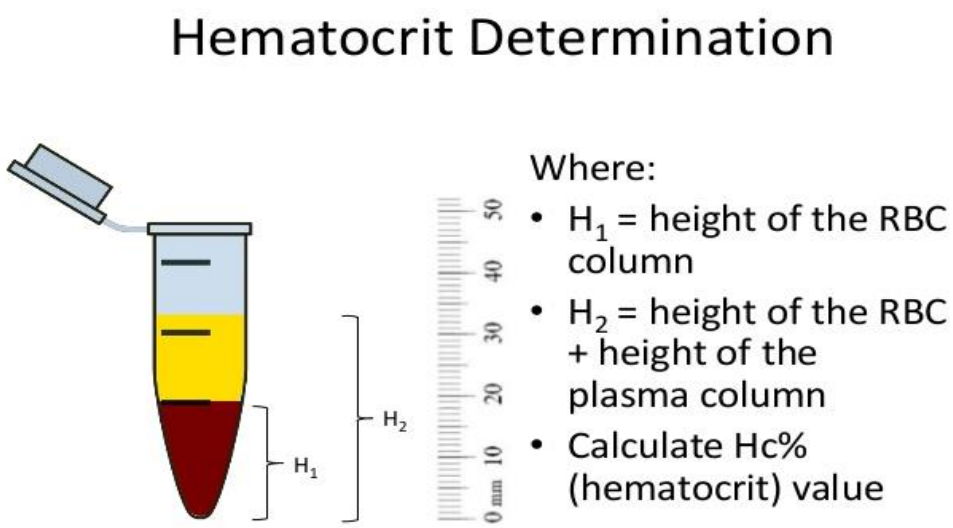


Figure 9: Hematocrit determination

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), amyelo proliferative 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 (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 80100 fL.

$$\text{MCV (femtoliter)} = \frac{\text{Hematocrit (\%)} \times 10}{\text{RBC count (millions/mm}^3\text{ blood)}}$$

Figure 10: Equation to determine the percent of hematocrit

Higher than Normal MCV

- In pernicious anemia (macrocytic), MCV can range up to 150femtolitres.
- An elevated MCV is also associated with alcoholism(as are an elevated GGT and a ratio of AST:ALT of 2:1).
- Vitamin B12and/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 femto litres.
- 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,
- 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.

$$MCV = \frac{PCV \times 1000}{\text{Erythrocyte count}} \text{ (Er) expressed in fentolitres}$$

$$MCH = \frac{\text{Haemoglobin value}}{\text{Erythrocyte count}} \frac{Hb}{Er} \text{ expressed in Picogrammes}$$

$$MCHC = \frac{Hb}{PCV \times 1000} \text{ expressed in g } L^{-1} \text{ and from the haematocrit value (PCV).}$$

Figure 11: Equations to determine MCV, MCH and MCHC

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.

1.5.5 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 nuclei, that are somewhat 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: 37×103mm3.

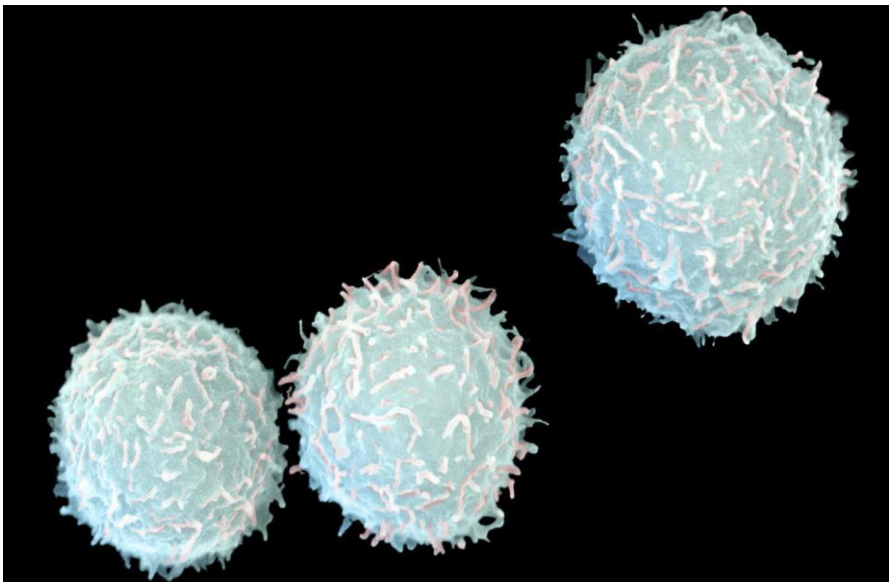


Figure 12: White Blood Cell

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 chemo toxic 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 1248 hours.

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).

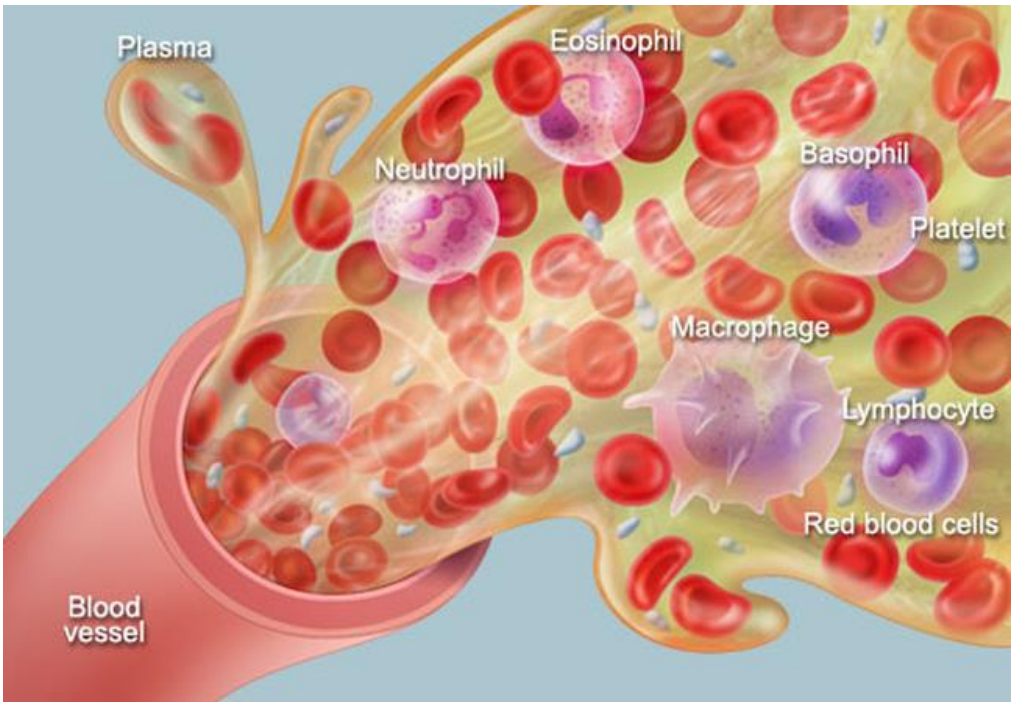


Figure 13: Various kinds of white blood cells

1.5.6 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.

Figure 14: Platelet

- The sticky surface of the platelets allow them to accumulate at the site of broken blood vessels to form a clot. This aids in the process of hemostasis ("blood stopping").
- Normal range of platelet: 1000-1600×103mm3 (Ganong, 2003).

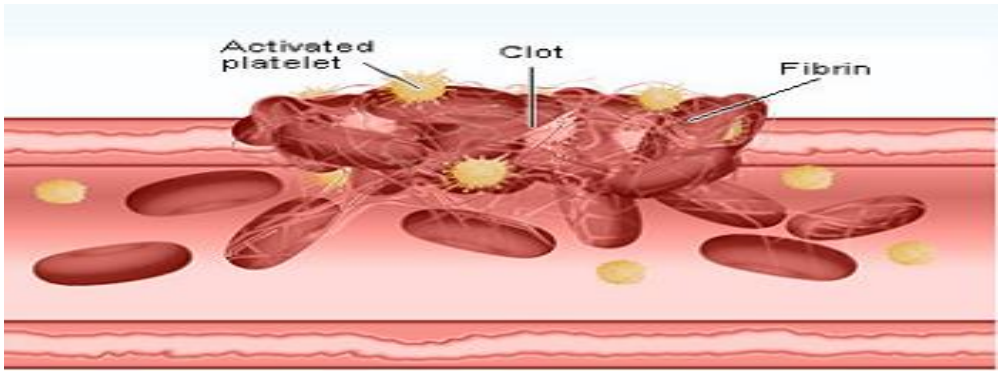


Figure 15: Platelet aggregation to form a blood clot

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(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

1.5.7 Hepatotoxicity

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

Several factors predispose the liver to xenobiotic toxicity:

- **Firstly**, for chemicals entering the body via the oral route, anatomical proximity to the GI-tract ensures the liver is the first port of call for ingested xenobiotics.
- **Secondly**, chemicals and nutrients are not the only substances that enter portal blood as it perfuse 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 bio activation-dependent hepatotoxicity (Philip and Burcham, 2014).

1.5.8 Liver

- The liver is avital organ of vertebrate sand 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 bio-chemicals necessary for digestion.

The liver is a gland and plays a major role in-

- metabolism with numerous functions in the human body
- regulation of glycogen storage
- decomposition of red blood cells
- protein synthesis, hormone production
- detoxification.
- It is an accessory digestive gland and produces bile, an alkaline compound which aids indigestion 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).

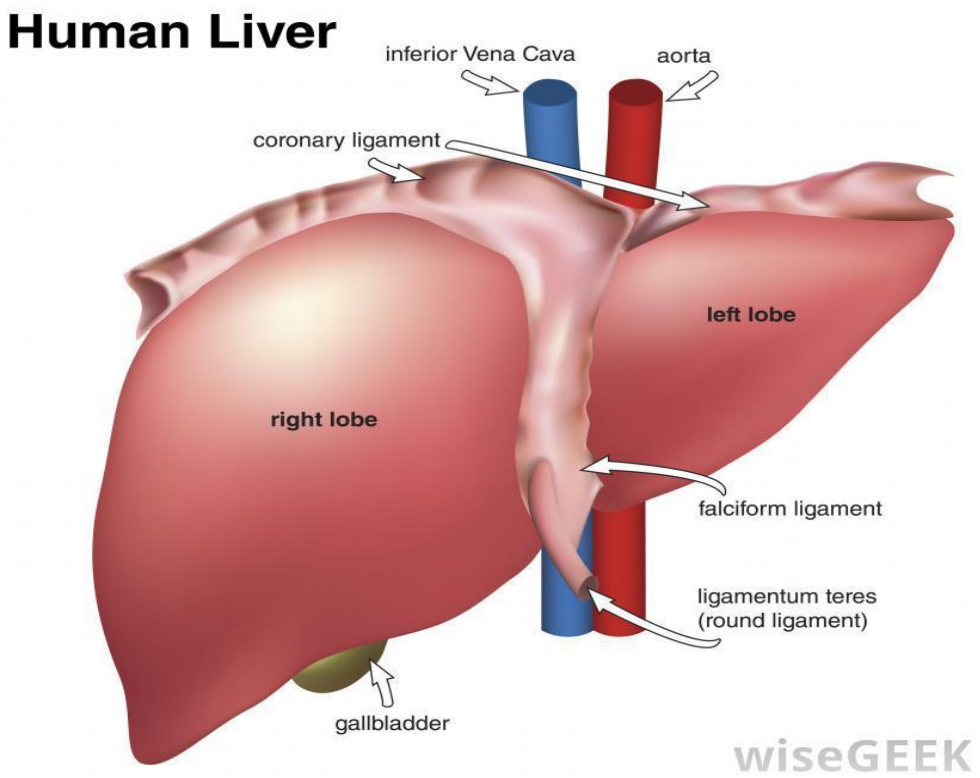


Figure 16: Human Liver

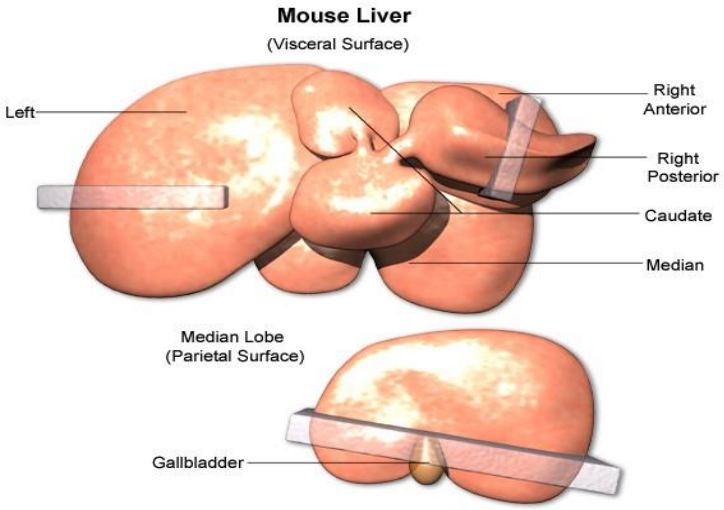


Figure 17: Mouse Liver

Functions

- The liver is considered a gland—an organ that secretes chemicals—because it produces bile, 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

1.5.9 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).

Uses of liver function test-

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

Table-3: **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 pre albumin, which is better at detecting acute changes (half-life of albumin and pre albumin 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.

Figure 18: 3D structure of Alkaline Phosphatase

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

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

Aspartate transaminase

AST, also called serum glutamic oxalo acetic 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).

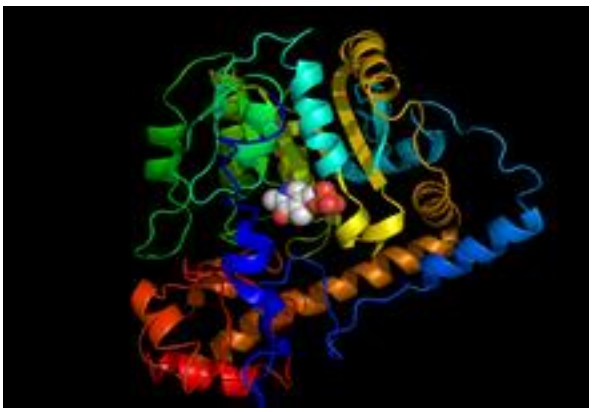


Figure 19: 3D structure of aspartate transaminase

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, acetaminophen, salicylates (aspirin).(Pratt, 2010).

Chapter 2 Introduction of Plant

2.1 Introduction of the plant

Medicinal plants possess various medicinal properties; have been serving as the major sources of therapeutic agents for maintenance of human health. These medicinal plants were used by the early human beings, as are done now, in a variety of forms, such as in the entire form, and as powders, pastes, juices, infusions and decoctions for the treatment of their various diseases and ailments. These various converted forms of the medicinal plants may therefore conveniently and genuinely called medicinal preparations or medicaments. This way, the medicinal plants formed an integral part of the health management practices and constituted important items of medicines used in the treatment of diseases from the very early days of human civilization.

Glycosmis pentaphylla belongs to the family Rutaceae. The genus Glycosmis of the family Rutaceae is represented by nearly 11 species. *Glycosmis pentaphylla*, is a shrub or small (1.5–5.0 m) tree widely distributed from India, Malaysia and Southern China to the Philippine Islands where it occurs in tropical forests at low altitudes. It is traditionally used for the treatment of fever, liver complaints and certain other diseases. The stems are widely used as a brush for cleaning the teeth. (Howlader et al, 2011)

2.1.1 Description of *Glycosmis pentaphylla*

Scientific name: *Glycosmis pentaphylla*(Retz) DC

Synonyms: *Glycosmis Arborea (Roxb.) A. DC, Glycosmis cochinchinensis* Pierre *ex* Engler

Family: Rutaceae

Bengali/Vernacular Name: Ashshaora, Datmajan, Matmati, Kawatuti, Aidali, Fatik, Ban Jamir; Motkila (Comilla).

Tribal Name: Hotiggira (Chakma); Si Ma Sere (Marma).

English Name: Toothbrush Plant, Motar tree.

Taxonimic Position

Kingdom: Plantae

Order: Sapindales

Family: Rutaceae

Sub Family: Aurantioideae

Tribe: Clauseneae

Genus: Glicosmis

Species: *Glycosmis pentaphylla (Glycosmis pentaphylla (Retz.) A. DC*, 2011)

2.2 Description:

An evergreen shrub. 0.9-1.8 m high. Leaves alternate, 3-7 foliolate, up to 18 cm long; leaflets 7.5-18 cm long, elliptic, rhomboid or ovate, aromatic when crushed. Flowers small, yellowish in terminal softly pubescent panicles, 10-30 cm long. Berry 1-1.8 cm long, ovoid, pale orange when ripe. *(Glycosmis pentaphylla (Retz.) A. DC*, 2011)

Figure 20: *Glycosmis pentaphylla*

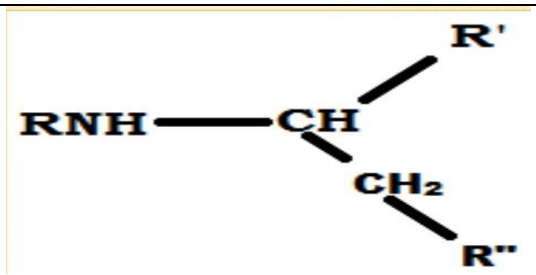
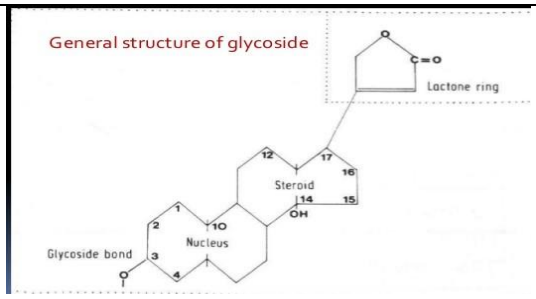
2.3 Geographical Distribution:

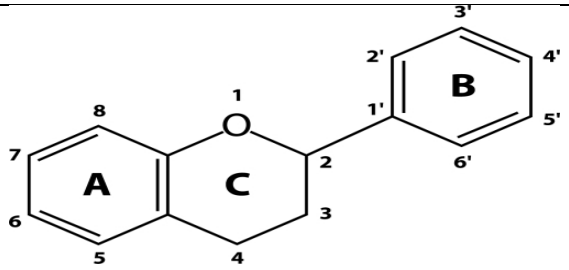
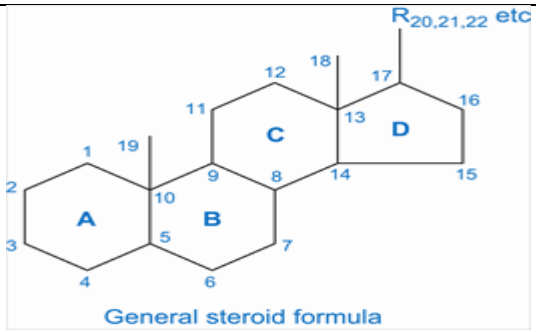
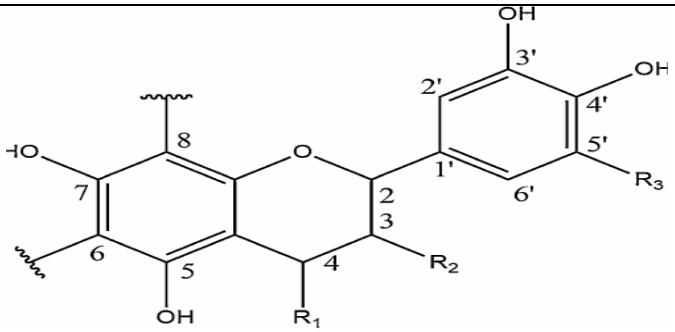
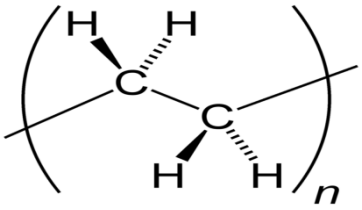
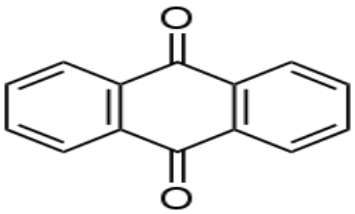
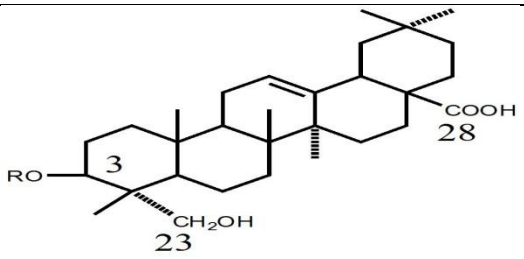
It is found in India, Sri Lanka, Myanmar, Bangladesh, Thailand, southern China, Indochina, possibly the Philippines, Peninsular Malaysia, Sumatra and Java.*(GLYCOSMIS PENTAPHYLLA (Retz.) A. DC*, 2011)

2.4 Chemical composition

- Air dried plant material yielded two fluoroquinoline bases, kokusaginine and skimmianine.
- Other alkaloids reported from the leaves include glycosine, arborine, glycosminine, arborinine (major), glycosamine, glycorine, glycosmicine and γ-fagarine. They also contain the triterpenes, arbinol and isoarbinol, arborinone, two isomeric terpene alcohols, myricyl alcohol, stigmasterol and β-sitosterol.
- Roots contain the carbazole alkaloids, glycozolicine, 3-formylcarbazole, glycosinine, glycozoline, glycozolidine, skimmianine, γ-fagarine and dictamine.
- Stems contain arborinine; other minor alkaloids also occur in this plant. The alkaloids arborine, arbornine, skimmianine, glycorine, glycophymine, glycophymoline, glycosmicine and glycomide have been isolated from the flowers. Glycoric acid has been isolated from the methanolic extract of the plant. (*Glycosmis pentaphylla (Retz.) A. DC*, 2011)

Table 2.1: Chemical composition of *Glycosmis pentaphylla*.

Phytochemicals	General Structure
Alkaloid	
Glycoside	

Flavonoid	
Steroid	
Tannin	
Resin	
Anthra quinine	
Saponin	

2.5 Medicinal Uses:

- The plant is used for cough, rheumatism, anaemia and jaundice.
- Leaf juice is given with sugar in empty stomach in the morning to eradicate ascaris. Young leaves along with the leaf juice of *Ananas sativus* is also given in the treatment of ascaris. The juice is also given in fever and liver complaints. Paste of leaves with ginger is used in eczema and skin affections.
- Roots are used in low fever. Leaf extract and crude alkaloid possesses antibacterial and antifungal properties. *(Glycosmis Pentaphylla (Retz.) A. DC*, 2011)

Chapter 3

Material and Method

3.1 Preparation of plant extraction

The whole part of the plant was dried in room temperature for approximately two weeks. Then the dried plants were taken into fine powder by using a grinding machine. Then the extraction process was done. At first 5 kg dried plant dust of *Glycosmis pentaphylla* was soaked in 26L methanol in 16 bottles. Then it was kept in room temperature for 7 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 7 days, the mixture was filtered. For filtration, white cotton cloth was used. After filtration two parts were obtained.

Figure 21: Herbarium sheet of *Glycosmis pentaphylla*

- The residue portion over the filter.

- The filtered part.

The filtrated part, which contains the substance soluble in methanol, poured into a 1000 round bottle flask, and then the flask was placed in a rotary evaporator. The evaporation was done at 53 degree Celsius temperature. The number of rotation per minute was selected as 85 RPM. The pressure of vacuum pump machine was 5 bars. The water flow through the distillation chamber was also provided in a satisfactory flow rate.

3.1.1 Crystal formation

After completing rotary, crystal was formed in a good amount. These crystals were clear and stable. These crystals were not soluble in polar and not polar solvent and intermediate solvent. Further investigation will be continued to know about these crystals.

Figure 22: Rotary filter for crystal formation

3.2 Experimental Animals

Swiss mice of either sex (25-35 g) 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 23: Swiss albino mice

3.3 CNS Activity Test

3.3.1 Materials for CNS Activity Test:

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

Chemical Agents Used in Test:

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

3.3.2 Doses Used in CNS Activity Test of the Extract:

i) Open Field Test:

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

ii) Hole Board Test:

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

3.4 Methods for CNS Activity Test:

To determine the 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 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 *Glycosmis pentaphylla* at the doses of 200,400 and 800 mg/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 24: 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 *Glycosmis pentaphylla* at the doses of 200,400 and 800mg/kg body weight orally. Each of the animal was transferred carefully to one corner of the field and the number of ambulation (expressed as the number of holes passed), head dipping and number of head poking was recorded for a period of 5 minutes at and post 30minutes intervals and were compared with the control animals.

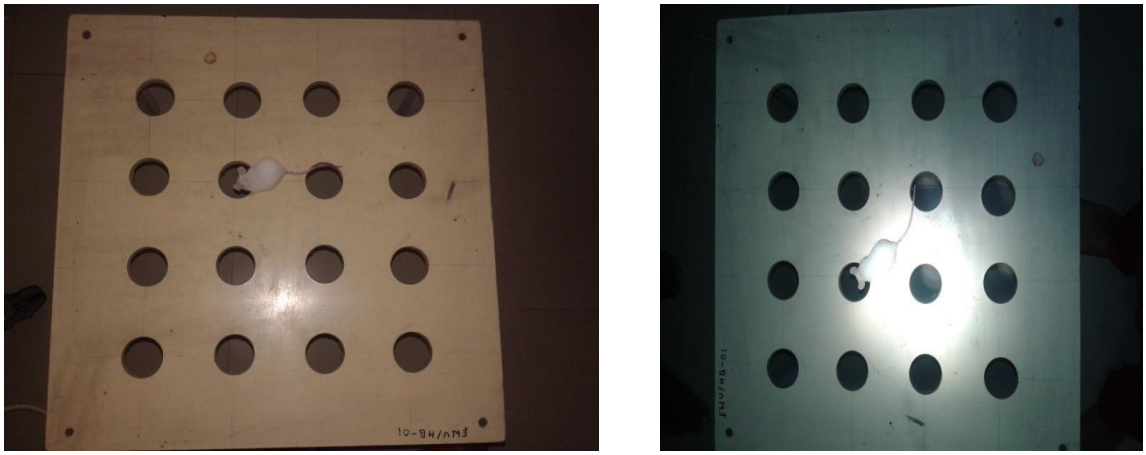


Figure 25: Hole Board Test

3.5 Toxicity Test

3.5.1 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

3.5.2 Chemical Agents Used in the Toxicity Test

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

3.5.3 Doses Used for Toxicological Activity of the Extract:

Chronic Toxicity Test:

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

3.6 Hematological parameters

Collected blood was used for the estimation of hemoglobin (Hb) content; red blood cell count (RBC) and white blood cell count (WBC).

3.7 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), serum total cholesterol, total protein, urea, uric acid and creatinine contents by using commercially available reagent kits.

3.8 Histopathological studies

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

3.9 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
Results and Discussion

4.1 Consequences of dosing

Methanolic extracts of *Glycosmis pentaphylla* at a dose of 200mg/kg, 400mg/kg and 800mg/kg of the crude extract were administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses. Diazepam was used as a positive control.

Figure 26: Consequences of dosing

After 60 days of dosing, two female mice (GP4F3 and GP8F8) were sacrificed and their hematology test and biochemical test for CBC and liver function were conducted and abnormal level of SGPT, SGOT and alkaline phosphate were obtained. The spleen of GP4F3 and GP8F8 numbered mice were found to be enlarged and damaged also.

4.2 CNS Activity Test of Methanolic Extract of *Glycosmis pentaphylla*

4.2.1 Open Field Test:

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

4.2.1.1 Total Number of Peripheral locomotion, Central locomotion and Leaning:

4.2.1.2 CNS activity of plant extract of *Glycosmis pentaphylla* 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	17.7±1.5	15.5±2.6	16.3±2.1	16.8±2.0	15.7±2.17
Crude extract of *Glycosmis pentaphylla*	200mg/kg	11±2.4	10±2.3	10.7±1.7	9.8±1.1	8.5±.93
Crude extract of *Glycosmis pentaphylla*	400mg/kg	7.1±2.9	13.9±1.8	11.2±1.7	10.6±1.3	6.3±1.2
Crude extract Of *Glycosmis pentaphylla*	800mg/kg	5.5±1.3	9.9±1.2	7.2±1.2	8±1	7.3±1.5
Positive control, Diazepam	1mg/kg	20.67±1.05	9.5±0.76	6.17±0.6	4.17±0.6	3.33±0.42

Each value is the mean ± SEM for 10 mice, *P<0.5; **P<0.01; ***P<0.001 compared with control. Data were analyzer by using One-Way ANOVA.

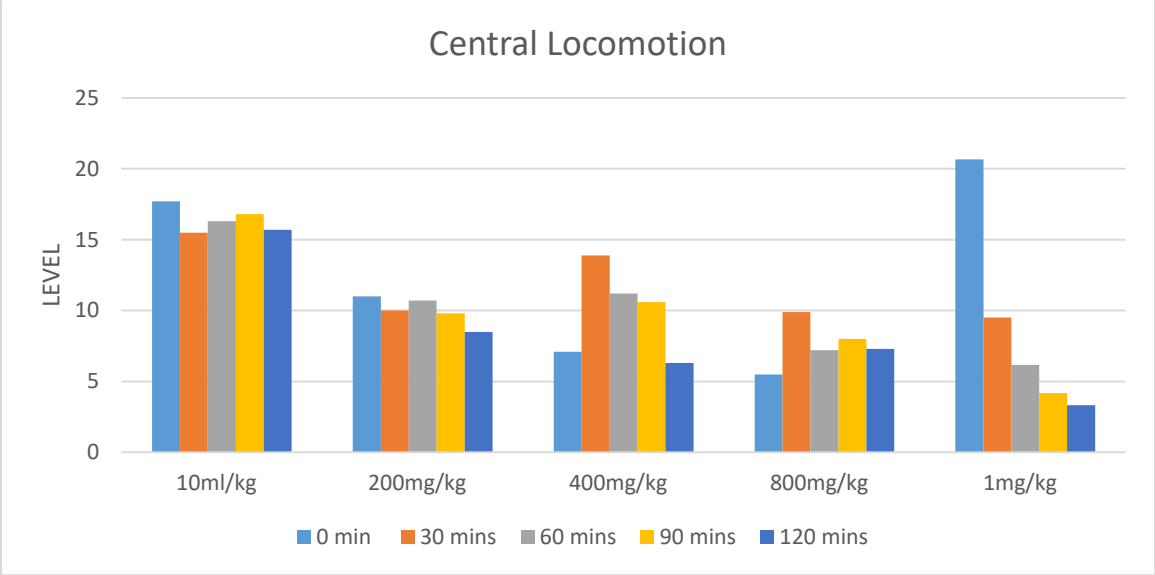


Figure 27: No. of central locomotion through graphical presentation

The graph showed that the central locomotion of the female mice after administering 200mg/kg, 400mg/kg and 800mg/kg of the methanolic extract of *Glycosmis pentaphylla* had been decreased. From that, it was observed that the extract had depressive effect on the mice. Then, the result was compared with the data obtained from the positive control, Diazepam (10ml/kg). After comparison, it was stated that the extract was not more depressive than Diazepam.

4.2.1.3 CNS activity of plant extract of *Glycosmis pentaphylla* 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	88.2±4.46	91.3±1.94	76.9±3.72	86.8±1.5	97.1±3.2
Crude extract of *Glycosmis pentaphylla*	200mg/kg	85.1±1.4	75.3±1.9	60.8±.3	61.8±2.84	37.2±1.5
Crude extract of *Glycosmis pentaphylla*	400mg/kg	75.4±1.4	80.6±2.7	62.1±1.4	54.2±2.8	39.2±1.9
Crude Extract of *Glycosmis pentaphylla*	800mg/kg	65.5±4.2	61.9±5.7	52±1.3	40.9±1.7	30.1±7
Positive control, Diazepam	1mg/kg	60.83±1.1	52.33±1.12	50.0±1.81	35.67±1.17	27.83±1.72

Each value is the mean ± SEM for 10 mice, *P<0.5; **P<0.01; ***P<0.001 compared with control. Data were analyzer by using One-Way ANOVA.

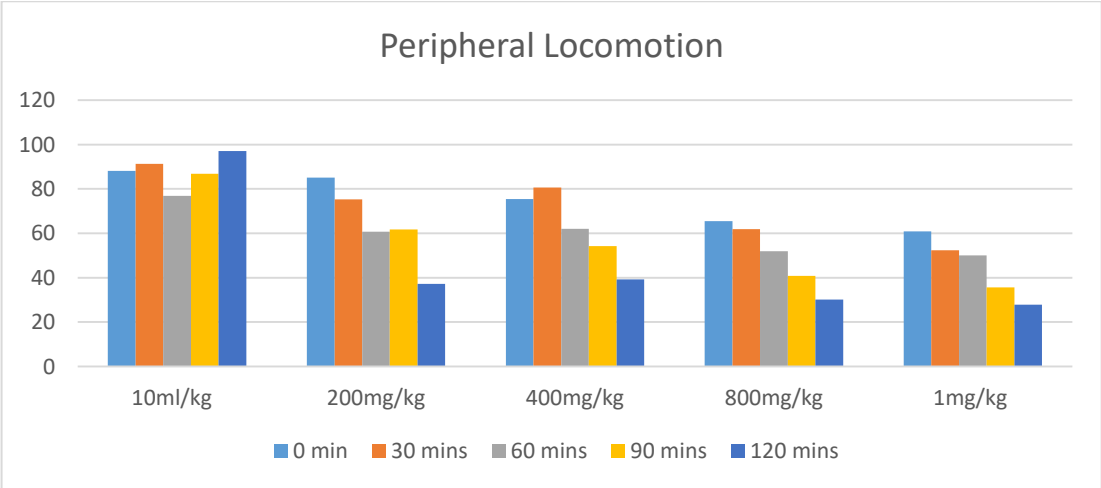


Figure 28: No. of peripheral locomotion through graphical representation

The graph showed that the peripheral locomotion of the female mice after administering 200mg/kg, 400mg/kg and 800mg/kg of the methanolic extract of *Glycosmis pentaphylla* had been decreased. The result was observed to have depressive effect on mice as the peripheral locomotion decreased from time to time. Then, the result was compared with the data obtained from the positive control, Diazepam (10ml/kg). the data did not show the extract to be more depressive than Diazepam.

4.2.1.4 CNS activity of plant extract of *Glycosmis pentaphylla* by Open Field Test (Leaning) in Mice.

Groups	Dose	No. of Leaning				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	12±2.1	13.3±2.4	11.5±1.8	12.6±1.8	14.1±2.28
Crude extract of *Glycosmis pentaphylla*	200mg/kg	9.4±1.6	10±.56	10.2±1.9	6.4±.92	5±1.33
Crude extract of *Glycosmis pentaphylla*	400mg/kg	5.3±1.9	11.4±2.6	9.3±2.3	5.8±1.2	7±.6
Crude extract of *Glycosmis pentaphylla*	800mg/kg	2.7±1.3	4.6±1.1	3.1±1	2.3±.44	4.7±1.1
Positive control, Diazepam	1mg/kg	22.17±1.08	4.83±0.31	3.17±0.31	2.1±0.33	2.8±0.48

Each value is the mean ± SEM for 10 mice, *P<0.5; **P<0.01; ***P<0.001 compared with control. Data were analyzer by using One-Way ANOVA.

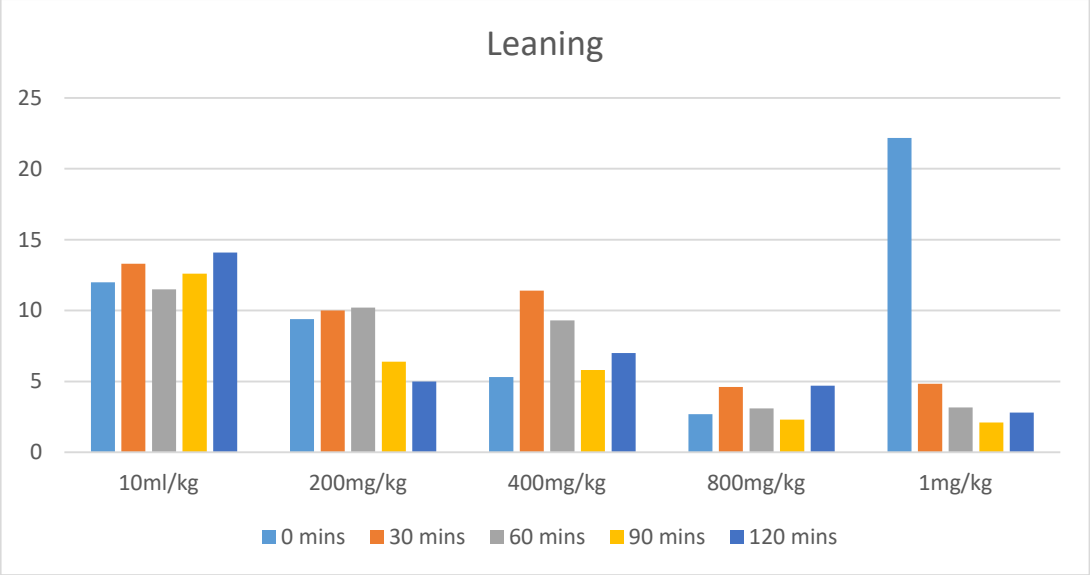


Figure 29: No. of leaning through graphical representation

The graph showed that the leaning of the female mice after administering 200mg/kg, 400mg/kg and 800mg/kg of the methanolic extract of *Glycosmis pentaphylla* had been decreased. The result was observed to have depressive effect on mice. Then, the result was compared with the data obtained from the positive control, Diazepam (10ml/kg). then it was clearly understood that the extract had depressive effect but it was not more depressant than Diazepam.

4.2.1.5 CNS Activity of plant extract of *Glycosmis pentaphylla* by Hole BoardTest in Mice.

Groups	Treatment	Dose	No. of Head Poking	No. of Head Dipping
Negative control	5% CMC	10ml/kg	68.5±1.48	25.17±1.01
Group-1	Crude extract of *Glycosmis pentaphylla*	200mg/kg	57.6±7.1	29.1±2.98
Group-2	Crude extract of *Glycosmis pentaphylla*	400mg/kg	49.9±6.5	26.8±3.95
Group-3	Crude extract of *Glycosmis pentaphylla*	800mg/kg	45.8±7.8	19.8±2.8
Positive control	Diazepam	1mg/kg	29.83±1.01	15.67±0.67

Each value is the mean ± SEM for 10 mice, *P<0.5; **P<0.01; ***P<0.001 compared with control. Data were analyzer by using One-Way ANOVA.

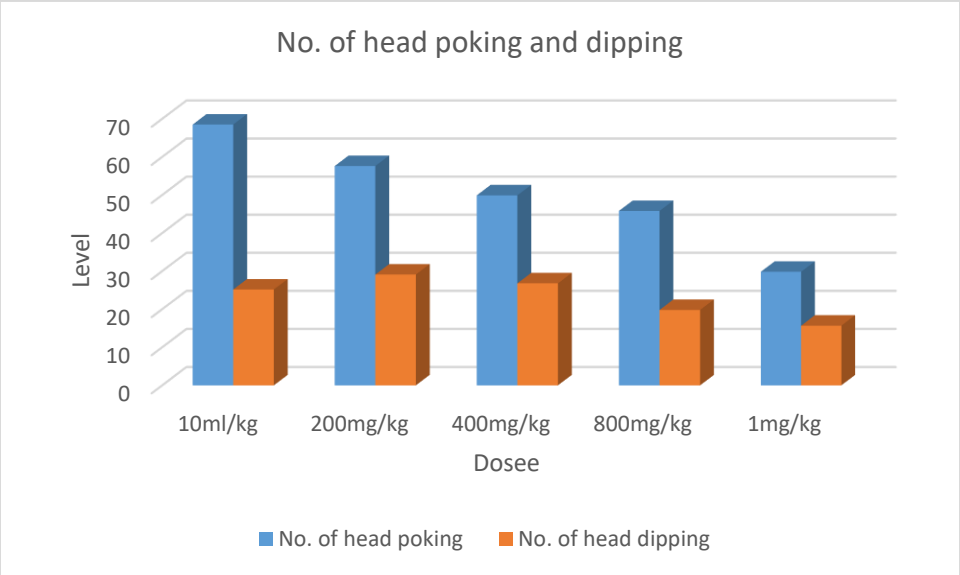


Figure 30: No. of head poking and dipping

The graph showed that the number of head poking and dipping of the female mice after administering 200mg/kg, 400mg/kg and 800mg/kg of the methanolic extract of *Glycosmis pentaphylla* had been decreased. The result then showed to be depressive. Then, the result was compared with the data obtained from the positive control, Diazepam (10ml/kg). The result obtained from the extract was not more depressive than Diazepam.

4.3.1 Chronic Toxicity Test:

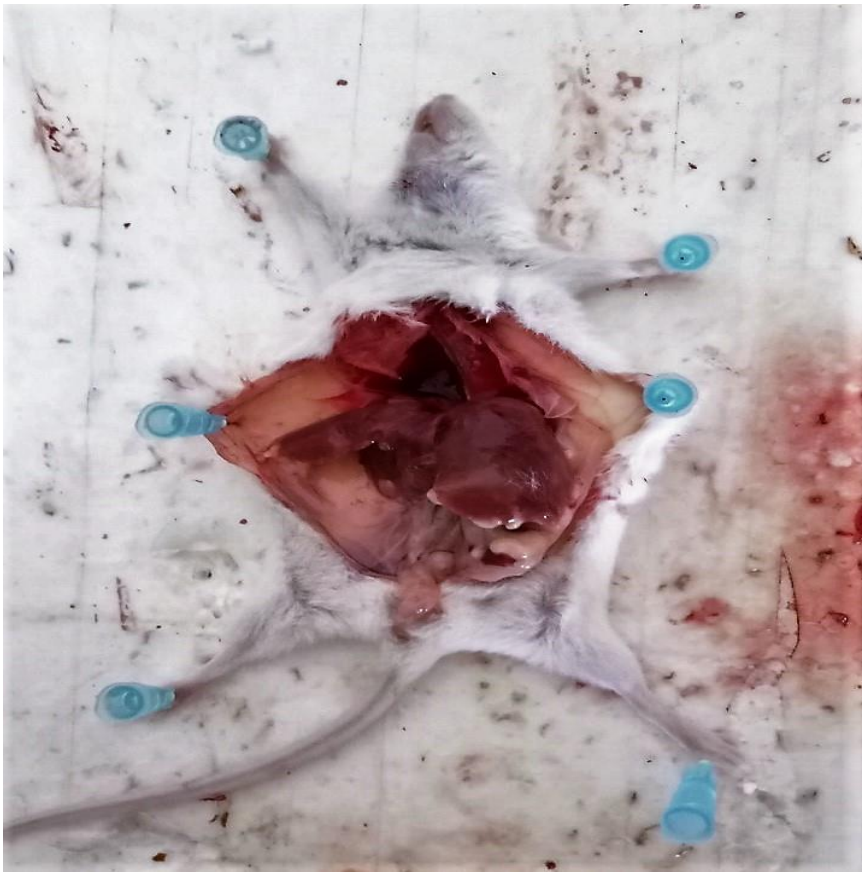


Figure 31: Sacrifice of a Swiss albino mice

CBC (Count Blood Cell) Test, Biochemical Test:

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

In the chronic study of methanolic extract of ***Glycosmis pentaphylla*** at a dose (200,400,800 mg/kg) to the mice, significant difference were not found in the erythrocyte and leucocytes values of both the treated and control mice. In which case, the administration of ***Glycosmis pentaphylla*** methanolic extract for a period of 90 days cannot induce significant aneamia. Though minor irregularities were observed mainly in the RBC, WBC, Neutrophil, Platelet, SGPT, SGOT and (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 resulted in the massive increase of the level of SGPT, SGOT and alkaline phosphate in the liver. And a number of mice died.

Table 4.5: Effect of *Glycosmis pentaphylla* on the different count of WBC (White Blood Cell)

Treatment group	Dose	Total WBC	Neutrophil (%)	Lymphocyte (%)	Monocyte (%)	Eosinophil (%)	Basophil (%)
Negative control group(Female)	10ml/kg	8.98	72	32	6	7	.99
Glycosmis pentaphylla (200mg)	200mg/kg	9.60	75	27	4.00	4.00	0
Glycosmis pentaphylla (400mg)	400mg/kg	10.055	73	27.5	5.00	4.00	0
Glycosmis pentaphylla (800mg)	800mg/kg		71	30	5.00	5.00	0

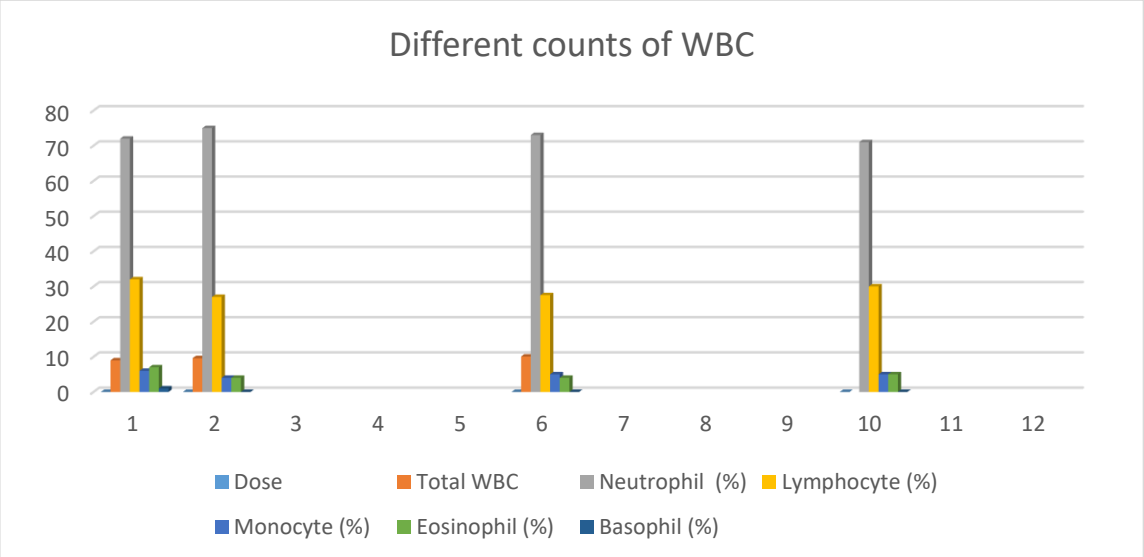


Figure 32: No. of different count of WBC (White Blood Test)

Table 4.6: Effect of *Glycosmis pentaphylla* on the Different count of RBC:

Treatment Group	Total RBC10^6/mm^3(n)	Hemo globin	HCT	MCV	MCH	MCHC	RDW
Negative control group(Female)	10.588	16.3	47.46	54.96	18.68	33.5	26.54
Glycosmis pentaphylla (200mg)	10.425	15.3	45.97	54.2	17.84	32.41	23.32
Glycosmis pentaphylla (400mg)	10.68	15.63	44.88	51.45	17.78	33.82	24.51
Glycosmis pentaphylla (800mg)	10.51	15.22	44.35	51.62	17.5	33.3	25.9

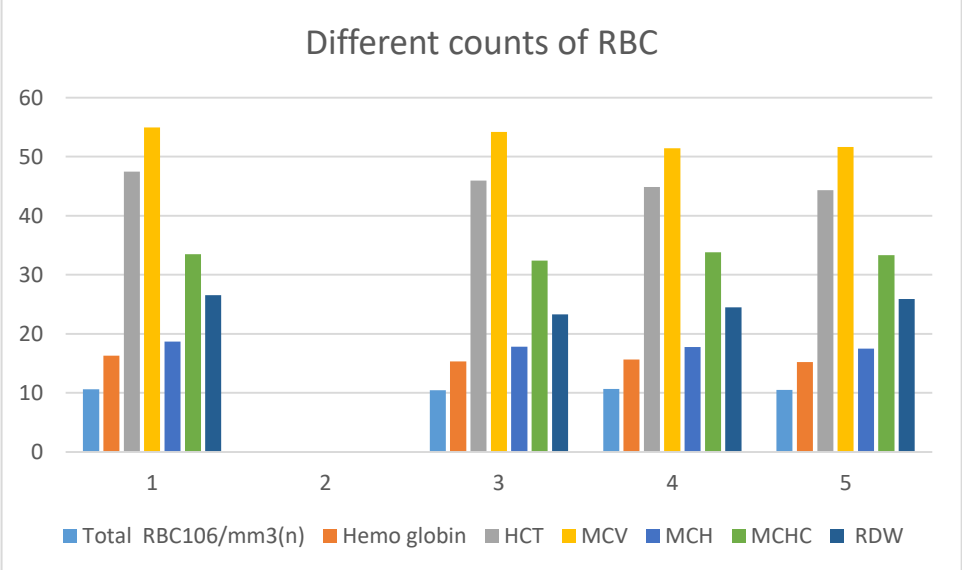


Figure 33: No. of different count of RBC (Red Blood Cell) through graphical representation

Table 4.7: Effect of *Glycosmis pentaphylla* on Platelet count on the CBC

Treatment group	Platelet
Negative control group(Male)	**863.4**
***Glycosmis pentaphylla* (200mg)**	**970.4**
***Glycosmis pentaphylla* (400mg)**	**1257.38**
***Glycosmis pentaphylla* (800mg)**	**767**

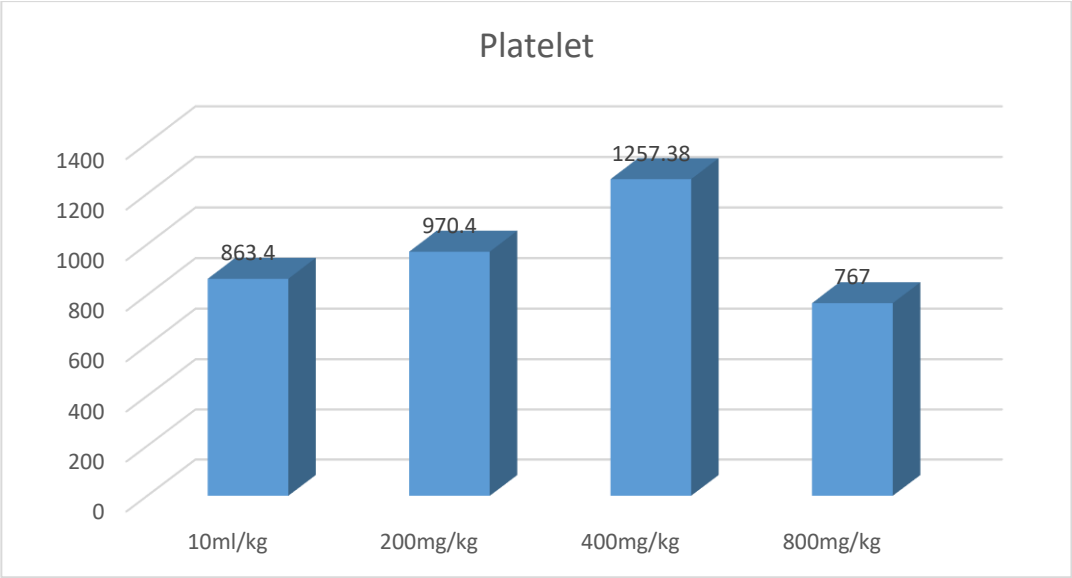


Figure 34: No. of platelets through graphical representation

Table 4.8: Effect of *Glycosmis pentaphylla* on the Liver Function Test

Treatment group	SGPT (U/L)	SGOT (U/L)	SALP (U/L)
Negative control group(Female)	42.6	30.8	128
Glycosmis pentaphylla (200mg)	52.5	35.14	237.14
Glycosmis pentaphylla (400mg)	58.25	53.37	229.75
Glycosmis pentaphylla (800mg)	87.25	59.25	259.75

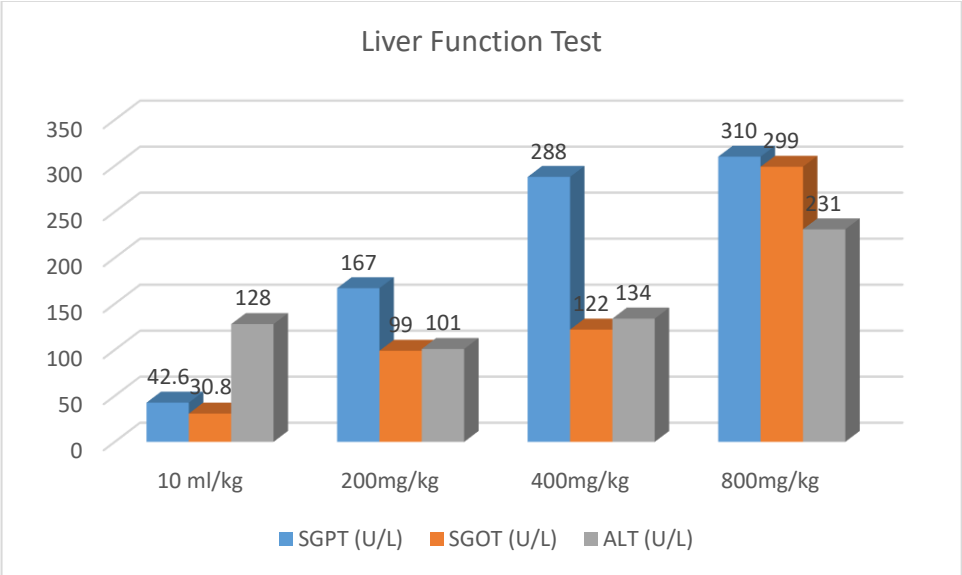


Figure 35: Graphical representation of Liver Function test

The graph showed that the SGPT, SGOT and Alkaline Phosphate level of the mice (200mg/kg, 400mg/kg and 800mg/kg) were increased drastically. The value exceeded the value of the

negative control, Diazepam (10ml/kg). It can be said that the liver and the heart were severely damaged. But furthers studies are needed to find out the specific problem.

Table 4.9: Effect of methanolic extract of *Glycosmis pentaphylla* on body weight in mice.

Treatment group	Dose	Initial body wt.	Final body wt.
Negative control group (Female)	**10ml/kg**	**29.6±0.6**	**38.4±1.93**
***Glycosmis pentaphylla* (200mg)**	**200mg/ kg**	**32.2±0.95**	**36.9±2.6**
***Glycosmis pentaphylla* (400mg)**	**400mg/ kg**	**30.8±1.9**	**41.7±1.2**
***Glycosmis pentaphylla* (800mg)**	**800mg/ kg**	**29.2±0.86**	**36.4±2.0**

Each value is the mean ± SEM for 10 mice, *P<0.5; **P<0.01; ***P<0.001 compared with control. Data were analyzer by using One-Way ANOVA.

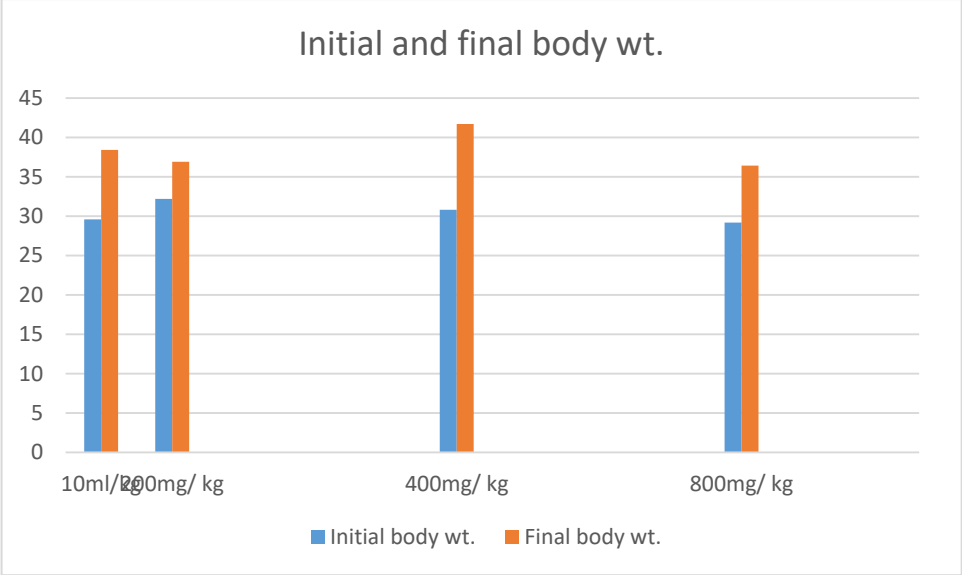


Figure 36: Graphical representation of the initial and final body weight of female mice.

Abnormal findings:

Figure 37: The damaged and enlarged spleen of GP4F3 mice

After sacrificing the GP4F3 mice, all the organs were separated and collected for histopathological studies. The size of the spleen was larger than its normal size. The spleen also appeared to be damaged. For further studies, this sample is given to the laboratory for histopathology test.

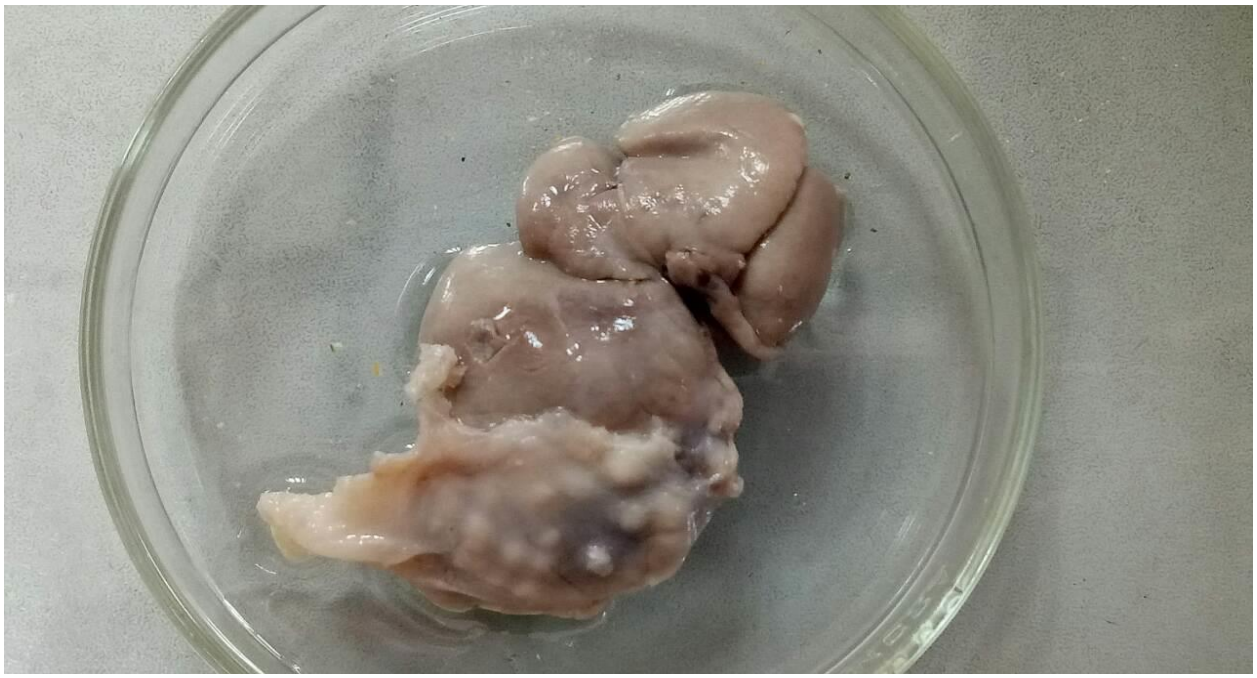


Figure 38: The attached and enlarged spleen and liver of GP8F8 mice

After sacrificing the GP8F8 mice, all the organs were separated and collected. But the spleen and the liver portion seemed to be attached to themselves that it could not be separated. The size of the spleen and the liver was also larger. To identify the specific problem, the sample is given to the laboratory for further histopathology studies.

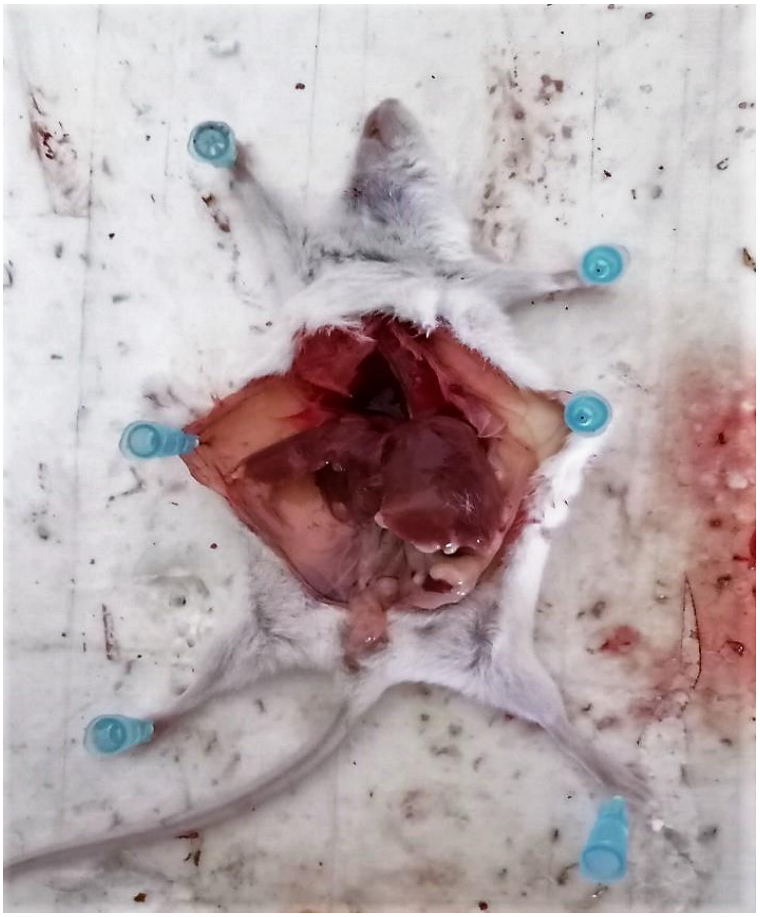


Figure 39: Sacrifice of a female mice where the internal organs are attached to one another

During the sacrifice of the GP8F10 mice, it was observed that the stomach, spleen, liver and intestine were glued to each other and they were not being able to be separated. So, ultimately, they were collected as a whole to let them undergo histopathology studies for further identification.

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

The aim of the study was also to investigate the possible toxicity of the plant *Glycosmis pentaphylla* 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 *Glycosmis pentaphylla* extract, in which significant difference were not found in RBC and different count of RBC. In which case, the administration of *Glycosmis pentaphylla* methanolic extract for a period of 90 days cannot induce significant aneamia.

Chapter 5
Reference

References:

Ansari, P., Azam, S., Hossain, A., Azad, T., Goswami, S. and Mondal, K.K., (2015). Potential Investigation of Anti-inflammatory Activity and Phytochemical Investigations of Ethanolic Extract of *Glycosmis pentaphylla* Leaves. *American Journal of Biomedical Research*, *3*(1), pp.6-8.

Beniddir, M.A., Le Borgne, E., Iorga, B.I., Loaëc, N., Lozach, O., Meijer, L., Awang, K. and Litaudon, M., (2014). Acridone Alkaloids from *Glycosmis chlorosperma* as DYRK1A inhibitors. *International Journal of natural products*, *77*(5), pp.1117-1122.

Bulbul, I.J. and Jahan, N., (2016). Study on Antioxidant and Antimicrobial Activities of Methanolic Leaf Extract of *Glycosmis pentaphylla* Against Various Microbial Strains. *Journal of Pharmacognosy and Phytochemistry*, *5*(4), pp.53.

Chan, Y.M. and Lim, A.L., (1970). The Reproductive and Seed Biology of *Glycosmis pentaphylla* (Rutaceae). *Malaysian Journal of Science*, *26*(2), pp. 12.

Chowdhury, M., Sultana, M.I., Tasneem, M.Z. and Al bin Jubair, A., *Phytochemical and Pharmacological Screening of Glycosmis pentaphylla* (Retz) A. DC. (Fam. Rutaceae).

Ebenezer, T., Kadamban, D., Shekhawat, M.S. and Justin Packia Jacob, S., (2013). Phytochemical Evaluation and Antioxidant Activity of *Glycosmis mauritiana* (Lam.) Tanaka Leaf Extract. *International Journal of Pharmaceutical Research Revised*, *2*, pp.16-23.

Gupta, N., Mb, A., Va., B., SKa, J. and Ja, D., (2011). Invitro Antioxidant Activity of Crude Extracts of The Plant *Glycosmis pentaphylla* Correa.

Howlader, M.A., Rizwan, F., Sultana, S., Rahman, M.R., Shams-Ud-Doha, K.M., Mowla, R. and Apu, A.S., (2011). Antimicrobial, Antioxidant and Cytotoxic Effects of Methanolic Extracts of Leaves and Stems of *Glycosmis pentaphylla* (Retz.). *International Journal of Applied Pharmaceutical Science*, *1*(8), pp.137.

Ito, C., Itoigawa, M., Sato, A., Hasan, C.M., Rashid, M.A., Tokuda, H., Mukainaka, T., Nishino, H. and Furukawa, H., (2004). Chemical Constituents of *Glycosmis arborea*: Three New Carbazole Alkaloids and Their Biological Activity 1. *Journal of Natural Products*, *67*(9), pp.1488-1491.

Khatun, M.C.S., Mia, M.R., Ali, M.A., Rahman, M.M., Begum, K. and Begum, K., (2013). Antidiabetic and Analgesic Effects of *Glycosmis pentaphylla* (Retz.) in Swiss Albino Mice. *Ibrahim Medical College Journal*, *6*(1), pp.21-26.

Murugesan, K., Chemical Composition and Antioxidant Activity of Essential Oil from *Glycosmis Pentaphylla*, pp. 15

Muthukrishnan, J. and Ananthagowri, B., (1994). Botanical Pesticides and Energetics in The Control of The Castor Semi looper *Achaea janata* Linnaeus (Noctuidae: Lepidoptera). *Phytophaga (Madras)*, *6*(2), pp.127-131.

Nayak, S.S., Jain, R. and Sahoo, A.K., (2011). Hepatoprotective Activity of *Glycosmis pentaphylla* Against Paracetamol-induced Hepatotoxicity in Swiss Albino Mice. *International Journal of Pharmaceutical Biology*, *49*(2), pp.111-117.

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.

Petchi, R.R. and Vijaya, C., (2012). Anti-diabetic and Anti-arthritic potential of *Glycosmis pentaphylla* Stem Bark in FCA Induced Arthritis and Streptozotocin Induced Diabetic Rats. *International Journal of Pharmaceutical Bio Science*, *3*(3), pp.328-336.

Poole, A. and Leslie, GB., (1989). A Practical Approach to Toxicological Investigations, (1st eds.), Great Britain. *Cambridge University press*, vol. 2, pp. 30-117.

Prakasia, P.P. and Nair, A.S., (2016). Evaluation of Invitro Antioxidant Potential of the Total Crude Alkaloid Extract of *Glycosmis Pentaphylla* (Retz.) Correa Leaves. *International Journal of Pharmacy and Pharmaceutical Sciences*, *8*(3), pp.85-91.

Shoja, M.H., Reddy, N.D., Nayak, P.G., Biswas, S., Srinivasan, K.K. and Rao, C.M., (2016). In vitro mechanistic and in vivo anti-tumor studies of *Glycosmis pentaphylla* (Retz.) DC against breast cancer. *Journal of Ethnopharmacology*, *186*, pp.159-168.

Sivakumar, M.M., (2015). *Pharmacognostical, Phytochemical and Pharmacological Screening of Root of Glycosmis pentaphylla (Retz.) DC* (Doctoral dissertation, Sri Ramachandra University), pp.5-6

Sreejith, P.S., Mascarenhas, R.R., Praseeja, R.J. and Asha, V.V., (2012). The Apoptosis Inducing Effect of *Glycosmis pentaphylla* (Retz.) Correa and Its Influence on Gene Expression in Hepatocellular Carcinoma Cell Line, *International Journal of Ethnopharmacology*, *139*(2), pp. 359-365.

Sreejith, P.S., Praseeja, R.J. and Asha, V.V., (2012). A Review on The Pharmacology and Phyto chemistry of Traditional Medicinal Plant, *Glycosmis pentaphylla* (Retz.) Correa. *Journal of Pharmaceutical Research.*, *5*, pp.2723-2728.