Pharmacological and Toxicological study of Medicinally Important Plant: *Drynaria quercifolia* (rhizome)

This Thesis Paper Submitted in Partial Fulfillment of the Requirement for the Degree

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DEDICATON

This Paper is dedicated to

My Beloved Parents

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation, entitled "**Pharmacological and Toxicological study of Medicinally Important Plant: *Drynaria quercifolia* (rhizome)**" is an authentic and genuine research work carried out by me under the guidance of Dr. Shamsun Nahar Khan, Associate Professor & Chairperson, Department of Pharmacy, East West University, Dhaka.

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ENDORSEMENT BY HEAD OF THE DEPARTMENT

This is to certify that the dissertation entitled "**Pharmacological and Toxicological study of Medicinally Important Plant: *Drynaria quercifolia* (rhizome)**" is a genuine research work carried out by Mohammed Ali, under the supervision of Shamsun Nahar Khan (Ph. D, Postdoc, Harvard University, Associate Professor & 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.

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CERTIFICATE

This is to certify that, the thesis on "**Pharmacological and Toxicological study of Medicinally Important Plant: *Drynaria quercifolia* (rhizome)**" submitted to Department of Pharmacy, East West University, Aftabnagar, Dhaka, in partial fulfillment of the requirements for the degree of Masters of Pharmacy (M. Pharm), was carried out by Mohammed Ali (ID # 2012-3-79-006) under our 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.

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Abstract

Historically Toxic Herbs like are used both good and bad way. Through there are some evidence that show the recent years of toxic consequence of herbal medicine increase day by day due to the lack of clinical , phytochemical , pharmacological and toxicological study. Medline search was undertaken to identify relevant literature using search terms including 'herbal', 'herbs', _toxicities‘, _cardiotoxicity‘, _hepatotoxicity‘, _nephrotoxicity‘, _hematologic toxicity‘ etc, which provide enough data on the numbers of adverse events are reported with the use of herbal products. *Drynaria quercifolia* is widely used as traditional medicine in our country to treat inflammation, rheumatism, headache, bone fracture and jaundice. But a reported journal on *Drynaria quercifolia* show its effectiveness as Pesticidal and pest repellency. So this study was evaluated the Sub Chronic Toxicity of methanolic extract of the rhizome of *Drynaria quercifolia*. Mainly three types of dose such as 200, 400 & 600 mg/kg were administered to the Swiss Albino Mice for 45 days. The different hematological test, biological estimation and histological microscopic observation were studied. This result exhibited potent total activity was expressed as an equivalent to control group where used 5% CMC solution. The results significant difference were not found in RBC but difference were found in WBC & different count of WBC (Neutrophil, Monocyte, Eosinophil,& Basophil) values of both treated & control mice. The result shows that increase toxic effect by increase dose such as (200,400 & 600) mg/kg and and decrease (Neutrophil, Monocyte, Eosinophil,& Basophil) values and also increase SGPT value. The histological status of the liver tissues of both the treated mice where normal cellular architecture with prominent central vain was shown which indicates, that the extract can cause damage to liver if used for maximum dose. The crude extract , n-haxen and dichloromethan fraction of methanolic extract of *Drynaria quercifolia* 200 mg/kg has been used to assess the analgesic properties by using animal models. The results suggest the presence of analgesic principles in *Drynaria quercifolia* that justifies its use for alleviating painful conditions In addition, for reviewing the sedative and anxiolytic properties of the methanolic extract of *Drynaria quercifolia* were investigated using mice behavioral models, such as Open Field for sedative property and Hole Board for anxiolytic potential, respectively. In Open Field Test, plant extract at the dose of 400mg/kg and 800 mg/kg both showed significant exploratory behavior. In the Hole Board Test in mice, plant extract at the dose of 400mg/kg and 800 mg/kg both showed anxiolytic activity.

Keywords: *Drynaria quercifolia* , Medicinal plant, Open Field, Hole Board, Analgesic, Writhing, Acute Toxicity, Sub-chronic Toxicity.

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1.1.1 Introduction

Phytotherapy has never stopped gaining in popularity. In low and middle income countries, it often represents the main, if not, the only therapeutic system to which majority of people are referred to for their primary health care .(WHO 2007) Toxicological tests have shown that many plants currently used are highly toxic when given either acutely or subchronically.(Bouguzza*et al.,* 2013)

The present study is an investigation of the possible toxicity of the *Drynaria quercifoloia* and a safety evaluation of the methanolicextract of the plant, focusing on its acute and chronic toxicity in mice, respectively. This chapter provides an overview of (1) toxicity of *Drynaria quercifolia* and the various definitions of key toxicity terms used in this report, (2) the current use and importance of herbal medicines, their traditional dosage forms and mode of administration of herbal medicines as well as some specific aspects of their toxicity. possible causes, prevalence and the evaluation of herbal toxicity, (3) its botany, main medicinal uses and dosage forms as well as its pharmacological activities and major chemical constituents, (4) with focus on their classification and biological activities and (5) with focus on its activities and aspects of its toxicity and the analytical techniques that are suitable for the determination of its levels in plant material and blood plasma.

1.1.2 Medicinal Plants

A considerable number of definitions have been proposed for medicinal plants. According to the WHO, “A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis.” When a plant is designated as ‘medicinal’, it is implied that the said plant is useful as a drug or therapeutic agent or an active ingredient of a medicinal preparation. “Medicinal plants may therefore be defined as a group of plants that possess some special properties or virtues that qualify them as articles of drugs and therapeutic agents, and are used for medicinal purposes” (Ghani A.,1998).

1.1.3Phytochemistry

Phytochemistry can be defined as the branch of biochemistry dealing with plants and plant processes. These are chemicals derived from plants. In a narrower sense the terms

are often used to describe the large number of secondary metabolic compounds found in plants. Many of these are known to provide protection against insect attacks and plant disease. They also exhibit a number of protective functions for human consumers. Phytochemistry also deals with the identification, biosynthesis and metabolism of chemical constituents of plants. It is the early class of organic chemistry.

1.1.4Primary Metabolites

Primary metabolites are involved in growth, development and reproduction of the organism. The primary metabolite is typically a key component in maintaining normal physiological processes; thus, it is often referred to as a central metabolite. Primary metabolites are typically formed during the growth phase as a result of energy metabolism, and are deemed essential for proper growth. Primary metabolites consist of various kinds of organic compounds, like carbohydrates, lipids, proteins and nucleic acids. Without primary metabolites key cellular cycles such as glycolysis, the Krebs cycle and the Calvin cycle is not possible, for that every plant kingdom contained those substances. This substance helps plants to take part in synthesis, assimilation and degradation of organic substances, sucrose and starch, structural components such as cellulose, information molecules such as DNA and RNA and pigments, such as chlorophyll are the main primary metabolite contained by the plants. Although these substances are key for the plant survival they also acts as precursors for the synthesis of secondary metabolites sometimes.

1.1.5 Secondary Metabolites

Secondary metabolites are substances which are produced by plants as defense chemicals. Their absence does not cause bad effects to the plants. They include alkaloids, phenolics, steroids, essential oils, lignins, resins and tannina etc.

Secondary metabolites are compounds bio synthetically derived from primary metabolites. Secondary metabolites or Secondary compounds are compounds that are not required for normal growth and development and are not made through metabolic pathways common to all plants. In plant kingdom they are limited to occurrence and may be restricted to a particular taxonomic group genus, species or family. Secondary metabolites are accumulated by plant cells in smaller qualities than primary

metabolites. Secondary metabolites are synthesized in specialized cells at particular developmental stages making extraction and purification difficult.

1.1.6 Photochemistry and Medicinal Plants

The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. That the medicinal actions of plants are unique to particular plant species or groups is consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct (Wink, 1999). This is in contrast to primary products, such as carbohydrates, lipids, proteins, heme, chlorophyll, and nucleic acids, which are common to all plants and are involved in the primary metabolic processes of building and maintaining plant cells (Kaufman *et al*., 1999; Wink, 1999).

1.1.7 Phytomedicine in Global Health Care

Phytomedicine or the use of herbal medicine with therapeutics properties has been around since the dawn of human civilization. A phytopharmaceutical preparation or herbal medicine is a manufactured medicine obtained exclusively from plants, either in the crude state or as pharmaceutical formulation. Although the industrial revolution and the development of organic chemistry resulted in a preference for synthetic products, World Health Organization (WHO) reports that between 70% and 95% of citizens in a majority of developing countries still rely on traditional medicine as their primary source of medication. In addition, of the top 150 proprietary drugs used in the United States of America (USA), 57% contain at least one major active compound currently or once derived from plants. In China traditional herbal preparation account for 30–50% of the total medicinal consumption. In Ghana, Mali, Nigeria and Zambia, the first line treatment for 60% of children with malaria is the use of herbal medicine. In San Francisco, London and South Africa, 70% of people living with HIV/AIDS use traditional medicine. Today the annual global market for herbal medicine stands at over US $ 60billion. (Mohamed *et al,*. 2012)

The ethnobotanical approach is actually one of several method that can be applied in choosing plants for pharmacological studies. It is estimated that 265,000 flowering

species grace the earth, less than half of 1% have been studied extensively for their chemical composition and medicinal value. In a world it is impossible to screen each of the remaining species for biological activity. For this reason, plants that have traditional medicinal value are usually used to screen & analyze their chemical constituents. However, it is interesting to note that the traditional uses of plants is one of the most successful criteria used by the pharmaceutical industry in finding new therapeutic agents for the various fields of biomedicine .

The well- established drugs listed below that were developed after scientists began to analyze the chemical constituents of plants used by traditional peoples for medicinal or other biological effects. For instance,Western researchers isolated Reserpine in 1952 from the climbing shrub *Rauvolfia serpentina* ,which has been employed in India for many centuries to treat snake bite and mental illness.

Table-1: List of Well-Established Drugs Developed from Natural Sources.

Drugs	Medical Use	Plant source
Aspirin	Reduces pain &inflammation	*Salix purpurea*
Codeine	Eases pain,suppresses couphing	*Papaver somniferum*
Ipecac	Induces vomiting	*Psychotria ipecacuanha*
pilocarpine	Reduces pressure in the eye	*Pilocarpus jaborandi*
Pseudoephedrine	Reduces nasal congestion	*Ephedra sinica*
Quinine	Combats malaria	*Cinchona pubescens*
Resrpine	Lower blood pressure	*Rauvolfia serpentine*
Scopolamine	Eases motion sickness	*Datura Stramonium*
Theophylline	Opens bronchial passages	*Camellia sinensis*
Vinblastine	Combats Hodgkin's diseases	Catharanthus roseus

This field is bringing forward new lead drug discoveries as well as safe and efficacious plant-based medicines. What chemists have been desperately seeking, Mother Nature has already plenty of stock. (Cox *et al*. 1994)

1.1.8 History of Traditional Medicine

Traditional medical practice remains the largest healthcare system in the world. The Hindu Kush Himalayas (Afghanistan, Bangladesh, Bhutan, China, India, Nepal and Pakistan) host the four largest traditional medicinal systems in the world, comprising of Ayurvedic medicine, Chinese medicine, Tibetan medicine and Unani medicine.

Ayurveda remains one of the most ancient traditional medicine systems and is still widely practiced in India, Sri Lanka and other countries with 400 000 registered ayurvedic practitioners in India alone. Atharvaveda (around 1200 B.C.), Charak Samhita and Sushrut Samhita (1000-500 B.C.) are the main literatures that give detailed description of over 700 herbs. Currently, over 1000 plants form the Ayurvedic Pharmacopoeia.

In China, herbal medicine continued it expansion from Sheng-Nongs Herbal Book (around 3000 B.C.) to the current updated inventory of medicinal plants used in Traditional Chinese Medicine (TCM) that includes 146 species of herbs/plants of which 492 species are cultivated and the remaining wild plants. Both the Ayurvedic and Chinese Traditional Medicine systems date back to ancient time, with an ever-expanding knowledge of plant properties and their medicinal qualities that were preserved in ancient texts.The Chinese government is one of the most active governments in the world in promoting the use of traditional medicine and integrating it with allopathic, conventional medicine. (Mohamed et al. 2012)

The Western version of herbal medicine began at the cradle of Western civilisation in Ancient Greece around the fifth century B.C. with the influence of accumulated medical knowledge from Egypt, Persia and Babylon. During the first century A.D., Dioscorides produced an influential herbal medicine text called De Materia Medica, which became a standard reference for Western practitioners for the next 1,500 years. Curiously, this book also included information on herbal remedies that had been used in Ayurvedic medicine for centuries. Around the same period, Galen of Pergamum formulated 130 antidotes and medicinal preparations (also known as galenicals) that may include up to 100 herbs and other substances.

The complexity involved in preparing these intricate medicinal prescriptions gave rise to the Galenic system that saw physicians as the ultimate authority in health care. It was around this time that traditional herbalists, with their "simple" remedies, began to be ousted from the mainstream medical system in Medieval Europe. Nevertheless, the knowledge of traditional herbal medicine was preserved by Catholic monks throughout the Middle Ages, with its practitioners still existing outside the mainstream system. Around the eighth century A.D.,Western herbal medicine began to be influenced by the herbal knowledge of Arab physicians who conducted extensive research on medicinal herbs found in Europe, Persia, India and the Far East. Later, with the discovery of the Americas in the fifteenth century A.D., a variety of the New World medicinal plants became available to Europeans.

Among the ancient knowledge of herbal medicine that came from the New World, the significant ones came from the Mexican traditional medicine, which combined the knowledge of four indigenous groups – Maya, Nahua, Zapotec and Mixe as well as the Inca civilisation. Medicinal plants continued to be the main source of products used for maintenance of health in Western conventional medicine until the nineteenth century when Friedrich Wohler accidentally synthesised urea in 1828. This first organic synthesis in human history ushered the age of synthetic compound. For the next 100 years, synthetic drugs became the mainstay of Western conventional medicine, with phytomedicine pushed into the shadows.

The role of herbal medicine started to decline after the 1960s as vast quantities of resources and money were used to promote synthetic medication. Besides this, advances in the human genome, increase knowledge of the structure and function of proteins and the notion that synthetic drugs are safer with fewer side effects (which does not necessarily be true) also contributed to the rise in the popularity of synthetic drugs. However, these advancements have several major constraints. The large number of possible new drug targets has already outgrown the number of existing compounds that could potentially serve as drug candidates and the field of chemistry has limitation when it comes to synthesising new drug structures.

In the last decade, herbal medicine has seen some form of revival, advancing at a greater pace in community acceptance of their therapeutics effects. (Mohamed et al. 2012)

1.1.9 Medicinal plants & Traditional Medicine Practice in Bangladesh

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

Use of these plants for therapeutic purposes has been in practice in this country since time immemorial. Continuous use of these plants as items of traditional medicine in the treatment and management of various health problems generation after generation has made traditional medicine an integral part of the culture of the people of this country. As a result, even at this age of highly advanced allopathic medicine, a large majority (75-80%) of the population of this country still prefer using traditional medicine in the treatment of most of their diseases even though modern medical facilities may be available in the neighbourhood.

Traditional medical practice among the tribal people is mainly based on the use of plant and animal parts and their various products as items of medicine.The medicaments, prepared from plant materials and other natural products sometimes also include some objectionable substances of animal origin. They are dispensed in a number of dosage forms like infusions, decoctions, pastes, moulded lumps, powders, dried pills, creams and poultices. Diets are strictly regulated. (Hussain, 2012)

1.2.1 Pain:

Pain is defined by) as an unpleasant sensory and emotional experience associated with potential tissue damage. (Bonica et al. 1979)The sensation of pain (nociception)is initiated in peripheral pain receptors (nociceptors) by stimuli which are sufficiently intense to cause tissue damage. Nociceptors and afferent pain fibers are present in skin, muscle, blood vessels, meninges, etc. The impulses generated as a result are conveyed to the CNS in special afferent pain fibers.Anti-nociception is termed as a reduction in the response of pain sensory system to noxious stimuli brought about by analgesics or anti-nociceptive drugs which are broadly classified into narcotics (opiates) and nonnarcotic (aspirin like) analgesics. The use of anti-inflammatory drugs and opioid

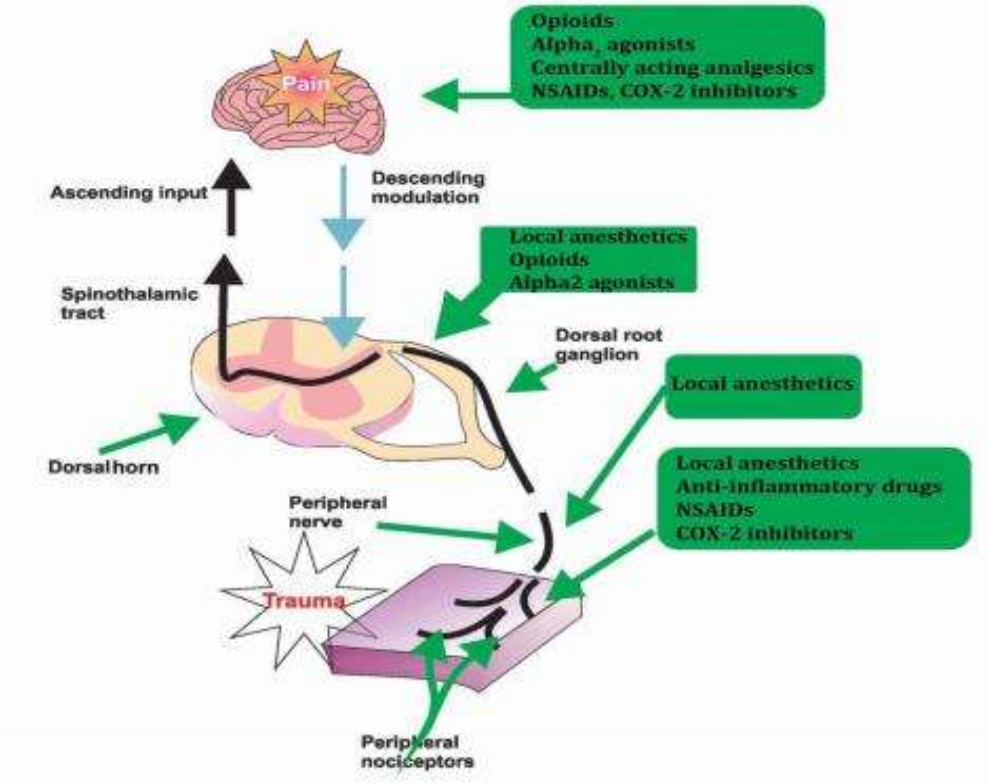

Figure. 1. The nociceptive pathway and prospective analgesia. (Kehlet et al, 1993)

analgesics helps to control the toxic chemical events leading to the sensitization of the peripheral nervous system and the further experience of pain (Okpako et al. 1991)**,**

In 1996 the International Association for the Study of Pain (IASP) defined pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described interms of such damage".The definition emphasizes both the physical and emotional nature of pain.

Pain is a multidimensional phenomenon with sensory, cognitive, affective, behavioural and spiritual components. Emotions(affective component),behavioural responses to pain (behavioural component), beliefs, attitudes, spiritual and cultural attitudes about pain and pain control (cognitive component) all alter the way that pain is experienced (sensory component) by modifying the transmission of noxious (unpleasant) stimuli to the brain (physiological component).

1.2.2. Classification of pain:

The four most commonly used systems for classify the pain are-

1. the pathophysiological mechanism of pain (nociceptive or neuropathic pain);
2. the duration of pain (chronic or acute, breakthrough pain);
3. the etiology (malignant or non-malignant);
4. The anatomic location of pain.

1.2.3 Pathophysiological classification

There are two major types of pain, nociceptive and neuropathic. Clinical distinction between nociceptive and neuropathic pain is useful because the treatment approaches are different.

Nociceptive pain:

Nociceptive pain arises when tissue injury activates specific pain receptors called nociceptors, which are sensitive to noxious stimuli. Nociceptors can respond to heat, cold, vibration, stretch stimuli and chemical substances released from tissues in response to oxygen deprivation, tissue disruption or inflammation.

This type of pain can be subdivided into somaticand visceralpain depending on the location of activated nociceptors.

• Somatic Pain:

Somatic painis caused by the activation of nociceptors in either surface tissues (skin, mucosa of mouth, nose, urethra, anus, etc. or deep tissues such as bone, joint, muscle or connective tissue. For example, cuts and sprains causing tissue disruption produce surface somatic pain while muscle cramps due to poor oxygen supply produce deep somatic pain.

Visceral Pain:

Visceral painis caused by the activation of nociceptors located in the viscera (the internal organs of the body that are enclosed within a cavity, such as thoracic and abdominal organs). It can occur due to infection, distension from fluid or gas, stretching or compression, usually from solid tumours.

Neuropathic Pain:

Neuropathic painis caused by structural damage and nerve cell dysfunction in the peripheral or central nervous system (CNS).Any process that causes damage to the nerves, such as metabolic,traumatic, infectious, ischaemic, toxic or immune-mediated pathological conditions, can result in neuropathic pain. In addition, neuropathic pain can be caused by nerve compression or the abnormal processing of pain signals by the brain and spinal cord.

Neuropathic pain can be either peripheral(arising as a direct consequence of a lesion or disease

affecting the peripheral nerve, the dorsal root ganglion or dorsal root) or central(arising as a direct consequence of a lesion or disease affecting the CNS). However, a clear distinction is not always possible.

1.2.4) Classification Based on Pain Duration:

A commonly used definition of acute pain is pain lasting less than 30 days, and a commonly used definition of chronicpain is pain lasting more than three months.

Acute Pain:

Acute pain is of sudden onset, is felt immediately following injury, is severe in intensity, but is usually short-lasting. It arises as a result of tissue injury stimulating nociceptors and generally disappears when the injury heals.

Chronic Pain:

Chronic pain is continuous or recurrent pain that persists beyond the expected normal time of healing. Chronic pain may begin as acute pain and persist for long periods or may recur due to persistence of noxious stimuli or repeated exacerbation of an injury. Chronic pain may also arise and persist in the absence of identifiable pathophysiology or medical illness. Chronic pain can can lead to distress, anxiety, depression, insomnia, fatigue or mood changes, such as irritability and negative coping behavior.

1.2.5) Etiological Classification:

Classification by etiology has little relevance to the mechanism and treatment of pain in children as categorization is commonly based on the underlying disease being malignant or non-malignant.

1.2.6Anatomical classification

Pain is often classified by body location (e.g. head, back or neck) or the anatomic function of the

affected tissue (e.g. myofascial, rheumatic, skeletal, neurological and vascular). However, location andfunction solely address the physical dimension and do not include the underlying mechanism. As such, although anatomical classifications can be useful for differential diagnoses, these classifications do not offer a framework for clinical management of pain.

1.2.7 Idiopathic Pain has no identifiable etiology. Examples are most headaches and recurrent abdominal Pain in specific disease conditions, such as cancer, HIV/AIDS and sickle cell disease, can be classified as mixed acute and/or chronic and may arise due to many of the causes. (World Health Organization, 2012)

1.2.8. Pain Pathway and Mechanism:

The experience of pain involves a series of complex neurophysiologic processes that reflect four distinct components:

- Transduction,
- Transmission,

- Perception, and
- Modulation.

Pain may occur in the absence of the occurrence of these four steps.

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1.2.8.1 Transduction of Pain

Transduction is the process by which a noxious stimulus is converted to an electrical impulse in sensory nerve endings. Transduction begins when the free nerve endings (nociceptors) of C fibres and A-delta fibres of primary afferent neurones respond to noxious stimuli. Nociceptors are exposed to noxious stimuli when tissue damage and inflammation occurs as a result of, for example, trauma, surgery, inflammation, infection, and ischemia.

Nociceptors are the specialised sensory receptors responsible for the detection of noxious (unpleasant) stimuli, transforming the stimuli into electrical signals, which are then conducted to the central nervous system. They are the free nerve endings of primary afferent Aδ and C fibres.
The nociceptors are distributed in the;

- somatic structures (skin, muscles, connective tissue, bones, joints);
- visceral structures (visceral organs such as liver, gastro-intestinal tract).
- The C fibre and A-delta fibres are associated with different qualities of pain.

1.2.8.2 Noxious Stimuli and Responses:

There are three categories of noxious stimuli:

- mechanical (pressure, swelling, abscess, incision, tumour growth);
- thermal (burn, scald);
- chemical (excitatory neurotransmitter, toxic substance, ischaemia, infection).

The cause of stimulation may be internal, such as pressure exerted by a tumour or external, for example, a burn. This noxious stimulation causes a release of chemical

mediators from the damaged cells including: prostaglandin, bradykinin, serotonin, substance P,potassium, histamine.

These chemical mediators activate and/or sensitise the nociceptors to the noxious stimuli. In order for a pain impulse to be generated, an exchange of sodium and potassium ions (de-polarisation and re-polarisation) occurs at the cell membranes. This results in an action potential and generation of a pain impulse. This process is called primary sensitization.

Table-2: Characteristics and Functions of C fibre and A-delta fibres.

C fibres	**A-delta fibres**
Characteristics: • Primary afferent fibres • Small diameter • Unmyelinated • Slow conducting	**Characteristics:** • Primary afferent fibres • Large diameter • Myelinated • Fast conducting
Receptor type: • Polymodal respond to more than one type of noxious stimuli: - • Mechanical, Thermal,Chemical	**Receptor type:** • High-threshold mechanoreceptors respond mechanical stimuli over a certain intensity.

1.2.8.3Transmission of Pain:

Transmission is the conduction of these electrical impulses to the CNS with the major connections for these nerves being in the dorsal horn of the spinal cord and thalamus with projections to the cingulate, insular and somatosensory cortexes.
The transmission process occurs in three stages. The pain impulse is transmitted-

- from the site of transduction along the nociceptor fibres to the dorsal horn in the spinal cord;
- from the spinal cord to the brain stem;
- through connections between the thalamus, cortex and higher levels of the brain.

The C fibre and A-delta fibres terminate in the dorsal horn of the spinal cord. The dorsal horn can be divided histologically into ten layer called Rexed laminae.Aδ and C fibres transmit information to nociceptive specific neurons in Rexed lamina I & II, in addition to projections to other laminae.

There is a synaptic cleft between the terminal ends of the C fibre and A-delta fibres and the nociceptive dorsal horn neurones (NDHN). In order for the pain impulses to be transmitted across the synaptic cleft to the NDHN, excitatory neurotransmitters are released, which bind to specific receptors in the NDHN. These neurotransmitters are: adenosine triphosphate,glutamate, calcitonin gene-related peptide,bradykinin,nitric oxide,substance P.

1.2.8.4 Ascending Tracts in The Spinal Cord:

The pain impulse is then transmitted from the spinal cord to the brain stem and thalamus via two main nociceptive ascending pathways. These are the spinothalamic pathway and the spinoparabrachial pathway.

• The spinothalamic tract: secondary afferent neurones decussate within a few segments of the level of entry into the spinal cord and ascend in the contralateral spinothalamic tract to nuclei within the thalamus. Third order neurones then ascend to terminate in the somatosensory cortex. There are also projections to the periaqueductal grey matter (PAG). The spinothalamic tract transmits signals that are important for pain localisation.

• The spinoreticular tract: fibres also decussate and ascend the contralateral cord to reach the brainstem reticular formation, before projecting to the thalamus and hypothalamus. There are many further projections to the cortex. This pathway is involved in the emotional aspects of pain.

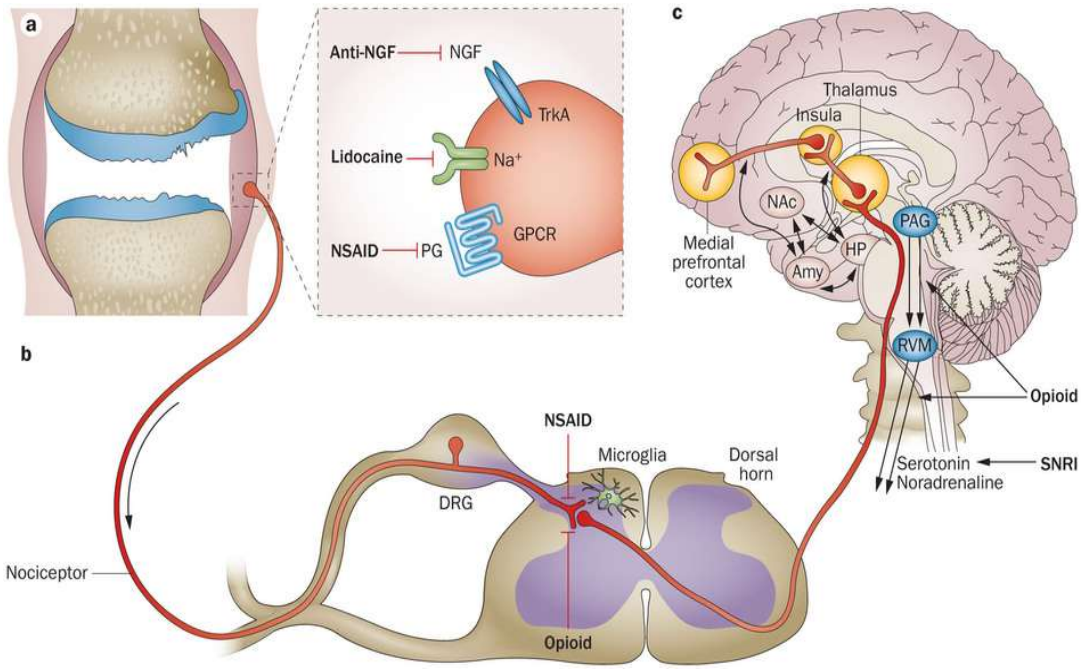


Figure-2: Pain Pathway and Its Blocking Mechanism.(Thomas.j et al 2013**)**

1.2.8.5 Modulation of Pain:

Modulation of pain is the process of altering pain transmission. It is likely that both inhibitory and excitatory mechanisms modulate pain (nociceptive) impulse transmission in the PNS and CNS.

The modulation of pain involves changing or inhibiting transmission of pain impulses in the spinal cord. The multiple, complex pathways involved in the modulation of pain are referred to as the descending modulatory pain pathways (DMPP) and these can lead to either an increase in the transmission of pain impulses (excitatory) or a decrease in transmission (inhibition).

1.2.8.10Inhibition of Pain Transmission:

There are mechanisms that act to inhibit pain transmission at the spinal cord level and via descending inhibition from higher centres. Such as-

i) Gate Control Theory of Pain:

The gate control theory of pain was proposed by Melzack and Wall in 1965 to describe a process of inhibitory pain modulation at the spinal cord level. It helps to explain why

when we bang our head, it feels better when we rub it. By activating Aβ fibres with tactile, non-noxious stimuli inhibitory interneurones in the dorsal horn are activated leading to inhibition of pain signals transmitted via C fibres.

ii) Descending Inhibition:

The periaqueductal grey (PAG) in the midbrain and the rostral ventromedial medulla (RVM) are two important areas of the brain involved in descending inhibitory modulation. Both these centres contain high concentrations of opioid receptors and endogenous opioids, which helps explain why opioids are analgesic. Descending pathways project to the dorsal horn and inhibit pain pain transmission. These pathways are monoaminergic,

Descending inhibition involves the release of inhibitory neurotransmitters that block or partially block the transmission of pain impulses, and therefore produce analgesia. Inhibitory neurotransmitters involved with the modulation of pain include:endogenous opioids (enkephalins and endorphins),serotonin (5-HT),norepinephirine (noradrenalin),gamma-aminobutyric acid (GABA),neurotensin, acetylcholine, oxytocin.

iii) Endogenous Pain Modulation:

Endogenous pain modulation helps to explain the wide variations in the perception of pain in different people as individuals produce different amounts of inhibitory neurotransmitters. Endogenous opioids are found throughout the central nervous system (CNS) and prevent the release of some excitatory neurotransmitters, for example, substance P, therefore, inhibiting the transmission of pain impulses.

1.2.8.11 Perception of Pain:

Pain perception is thought to occur at the thalamus with the cortex being important for discrimination of specific sensory experiences.

Perception of pain is the end result of the neuronal activity of pain transmission and where pain becomes a conscious multidimensional experience. The multidimensional experience of pain has affective-motivational, sensory-discriminative,emotional and behavioural components.When the painful stimuli are transmitted to the brain stem and thalamus, multiple cortical areas are activated and responses are elicited.

These areas are:

i)The Reticular System:

This is responsible for the autonomic and motor response to pain and for warning the individual to do something, for example, automatically removing a hand when it touches a hot saucepan. It also has a role in the affective-motivational response to pain such as looking at and assessing the injury to the hand once it has been removed form the hot saucepan.

ii) Somatosensory cortex:

This is involved with the perception and interpretation of sensations. It identifies the intensity, type and location of the pain sensation and relates the sensation to past experiences, memory and cognitive activities. It identifies the nature of the stimulus before it triggers a response, for example, where the pain is, how strong it is and what it feels like.

iii) Limbic System:

This is responsible for the emotional and behavioural responses to pain for example, attention, mood, and motivation, and also with processing pain and past experiences of pain. (Wood, 2008)

1.2.9, Evaluation of Pain:

Patient Description & History

A doctor's first step in evaluating a patient's pain is obtaining a detailed description of the pain, including:

- Severity
- Timing (time of day; continuous or intermittent)
- Location in the body
- Quality (piercing, burning, aching, etc.)
- Factors that relieve the pain or make it worse (temperature or humidity; body position or level of activity; foods or medications; emotional stress, etc.)
- Its relationship to mood swings, anxiety, or depression

Physical Examination

A thorough physical examination is essential in identifying the specific disorders or injuries that are causing the pain.

Special Tests

Although there are no laboratory tests or imaging studies that can demonstrate the existence of pain as such or measure its intensity directly, the doctor may order special tests to help determine the causes of the pain.

These studies may include one or more of the following:

Imaging Studies:

Usually X rays or magnetic resonance imaging's (MRIs). These studies can detect abnormalities in the structure of bones or joints, and differentiate between healthy and diseased tissues.

Neurological Tests:

These tests evaluate the patient's movement, gait, reflexes, coordination, balance, and sensory perception.

Electrodiagnostic Tests:

These tests include electromyography (EMG), nerve conduction studies, and evoked potential (EP) tests. In EMG, the doctor inserts thin needles in specific muscles and observes the electrical signals that are displayed on a screen. This test helps to pinpoint which muscles and nerves are affected by pain. Nerve conduction studies are done to determine whether specific nerves have been damaged. EP tests measure the speed of transmission of nerve impulses to the brain by using two electrodes, one attached to the patient's arm or leg and the other to the scalp.

Thermography:

This is an imaging technique that uses infrared scanning devices to convert changes in skin temperature into electrical impulses that can be displayed as different colors on a computer monitor. Pain related to inflammation, nerve damage, or abnormalities in skin blood flow can be effectively evaluated by thermography.

Psychological Tests:

Such instruments as the Minnesota Multiphasic Personality Inventory (MMPI) may be helpful in assessing hypochondriasis and other personality traits related to psychogenic pain. (Rebecca J. Frey, 2004)

1.2.10 Treatment of Pain:

Analgesic drugs:

The term “Analgesic” is derived from two Greek words- “an”means "without" and “algos” means "pain".Analgesic, any drug that relieves pain selectively without blocking the conduction of nerve impulses, markedly altering sensory perception, or affecting consciousness.

Analgesics may be classified into two types-

1) Non-opioids .
2) Opioids.

1) **Non-opioids** also referred to as non-narcotic, peripheral, mild & antipyretic agents.Nonopiod drugs are used to treat mild to moderate pain. Nonopiods are classified into three categories:

i) Salicylates:

Aspirin is one of these types, used to treat mild to moderate pain, inflammation, fever, arthritis and reduce blood clotting. Aspirin is one of the oldest drugs and is highly effective.Acetylsalicylic acid (ASA, aspirin) is broken down into acetic acid and salicylic acid. Salicylates work by blocking prostaglandin synthesis in the peripheral nerves & the hypothalamus portion of the brain.They have the analgesic,anti-inflammatory& anti-pyratic properties

For example: Aspirin,Choline salicylate, Diflunisal, Magnesium salicylate ,Sodiumsalicylate.

Mechanism of Action of Aspirin:

The inflammatory response involves many compounds, including prostaglandins, histamine, leukotrienes, and thromboxane. The most important of these with regard to aspirin and SA are the prostaglandins, which are pro-inflammatory compounds, produced from arachidonic acid by a class of enzymes called cyclooxygenases (COX) (Vane & Botting, 1987). Aspirin works by two mechanisms. First, it chemically alters the COX-2 enzyme responsible for production of prostaglandins by donating an acetyl group to a serine residue. Secondly, it inhibits transcription of the COX-2 mRNA by an unknown mechanism (Xu et al., 1998). When the COX-2 gene is not transcribed, the COX-2 enzyme is not produced, and prostaglandins are not synthesized, reducing the inflammatory response. SA itself probably works through transcriptional regulation.

Side Effects of Aspirin

Serious side effects:

- Black, bloody, or tarry stools
- Coughing up blood or vomit that looks like coffee grounds
- Severe nausea, vomiting, or stomach pain
- Fever lasting longer than 3 days
- Swelling, or pain lasting longer than 10 days
- Hearing problems, ringing in your ears.

Less serious side effects may include:

- Upset stomach, heartburn;
- Drowsiness; or
- Headache**.** (Morley, 1978)

ii) NSAIDs or Non steroidal anti-inflammatory drugs:

NSAIDs are used to treat pain and are similar to aspirin in the mechanism of action, pharmacological effects, and adverse reactions. NSAIDs are available in

nonprescription & prescription strengths. They are also used to treat rheumatoid arthritis, osteoarthritis, dysmenorrhea, and additional inflammatory diseases. NSAIDs work by blocking prostaglandin synthesis in the peripheral nerves & the hypothalamus portion of the brain. They have the analgesic, anti-inflammatory,anti-coagulant & anti-pyratic properties.

For example: Etodolac, Ibuprofen, Ketoprofen, Naprosyn

A newer group of NSAIDs, which are sometimes called "superaspirins" because they can be given in higher doses than aspirin without causing stomach upset or bleeding, are known as COX-2 inhibitors. The COX-2 inhibitors include celecoxib, rofecoxib, and valdecoxib.

Mechanism of Action of NSAID:

Traditionally, the analgesic action of nonsteroidal anti-inflammatory drugs (NSAIDs) has been explained on the basis of their inhibition of the enzymes that synthesise prostaglandins. However, it is clear that NSAIDs exert their analgesic effect not only through peripheral inhibition of prostaglandin synthesis but also through a variety of other peripheral and central mechanisms. It is now known that there are 2 structurally distinct forms of the cyclo-oxygenase enzyme (COX-1 and COX-2). COX-1 is a constitutive member of normal cells and COX-2 is induced in inflammatory cells. Inhibition of COX-2 activity represents the most likely mechanism of action for NSAID-mediated analgesia, while the ratio of inhibition of COX-1 to COX-2 by NSAIDs should determine the likelihood of adverse effects. In addition, some NSAIDs inhibit the lipoxygenase pathway, which may itself result in the production of algogenic metabolites. Interference with G-protein-mediated signals transduction by NSAIDs may form the basis of an analgesic mechanism unrelated to inhibition of prostaglandin synthesis. These is increasing evidence that NSAIDs have a central mechanism of action that augments the peripheral mechanism. This effect may be the result of interference with the formation of prostaglandins within the CNS. Alternatively, the central action may be mediated by endogenous opioid peptides or blockade of the release of serotonin (5-hydroxytryptamine; 5-HT). A mechanism involving inhibition of excitatory amino acids of N-methyl-D-aspartate receptor activation has also been proposed. (Carin et al. 2006)

Side Effects of NSAID:

NSAIDs are associated with several side effects. The frequency of side effects varies among NSAIDs. The most common side effects are

- Nausea, vomiting
- Diarrhea, constipation
- Decreased appetite
- Rash
- Dizziness
- Headache, and drowsiness
- Fluid retention, leading to edema.
- The most serious side effects are kidney failure, liver failure, ulcers and prolonged bleeding after an injury or surgery. (Omudhome Ogbru, 2010)

iii) Acetaminophen

Acetaminophen is used to treat pain such as headaches, dental pain, dysmenorrhea,myalgias, neuralgias and fever particularly in patients who cannot take aspirin.It has analgesic,and anti-inflammatory effects.

2) Opioids:

Opioids are also called narcotic, central or strong agents. The word opioid refers to a morphine-like compound that affects the opiod receptors along the pain-analgesia pathway of the central nervous system , thereby reducing pain sensation. Narcotic or opiod drugs are used to treat moderate to severe pain that does not respond to nonopiod drugs.

Opiods are classified into three categories based on their opiate receptor activity:

Agonist: For example: Codeine, Hydrocodone, Hydromorphone, Meperidine, Morphine, and Oxycodone.

Mixed agonist: For example: Buprenorphine.

Antagonist: For example:Nalbuphine, Nalorphine, Naloxone, Pentazcine.(Richard *et al.* 2007)

Complementary & Alternative (CAM) Approaches

CAM therapies that are used in pain management include:

Acupuncture: Studies funded by the National Center for Complementary and Alternative Medicine (NCCAM) since 1998 have found that acupuncture is an effective treatment for chronic pain in many patients. It is thought that acupuncture works by stimulating the release of endorphins, the body's natural painkillers.

Exercise: Physical exercise stimulates the body to produce endorphins.

Yoga: Practiced under a doctor's supervision, yoga helps to maintain flexibility and range of motion in joints and muscles. The breathing exercises that are part of a yoga practice also relax the body.

Prayer & meditation: The act of prayer by itself helps many people to relax. In addition, prayer and meditation are ways to refocus one's attention and keep pain from becoming the center of one's life.

Naturopathy: Naturopaths include dietary advice and nutritional therapy in their treatment, which is effective for some patients suffering from chronic pain syndromes.

Hydrotherapy: Warm whirlpool baths ease muscular and joint pain.

Music Therapy: Music therapy may involve listening to music, making music, or both. Some researchers think that music works to relieve pain by temporarily blocking the "gates" of pain in the dorsal horn of the spinal cord, while others believe that music stimulates the release of endorphins. (Rebecca J. Frey,2004)

1.3.1.1Nervous System:

The human nervous system is perhaps the most complex system of any organism. The human brain alone contains over 100 billion nerve cells, and each nerve cell can have up to 10,000 connections to other nerve cells. This means that a nerve impulse—an electrochemical signal to or from the brain could travel along 10^{15} possible routes. The

nervous system has two major divisions: the central nervous system (CNS) and the peripheral nervous system (PNS).

Early researchers made this distinction based on where nervous tissue was located in the body centrally or away from the centre (peripherally). Together, the central nervous system and the peripheral nervous system control sensory input, integration, and motor output.

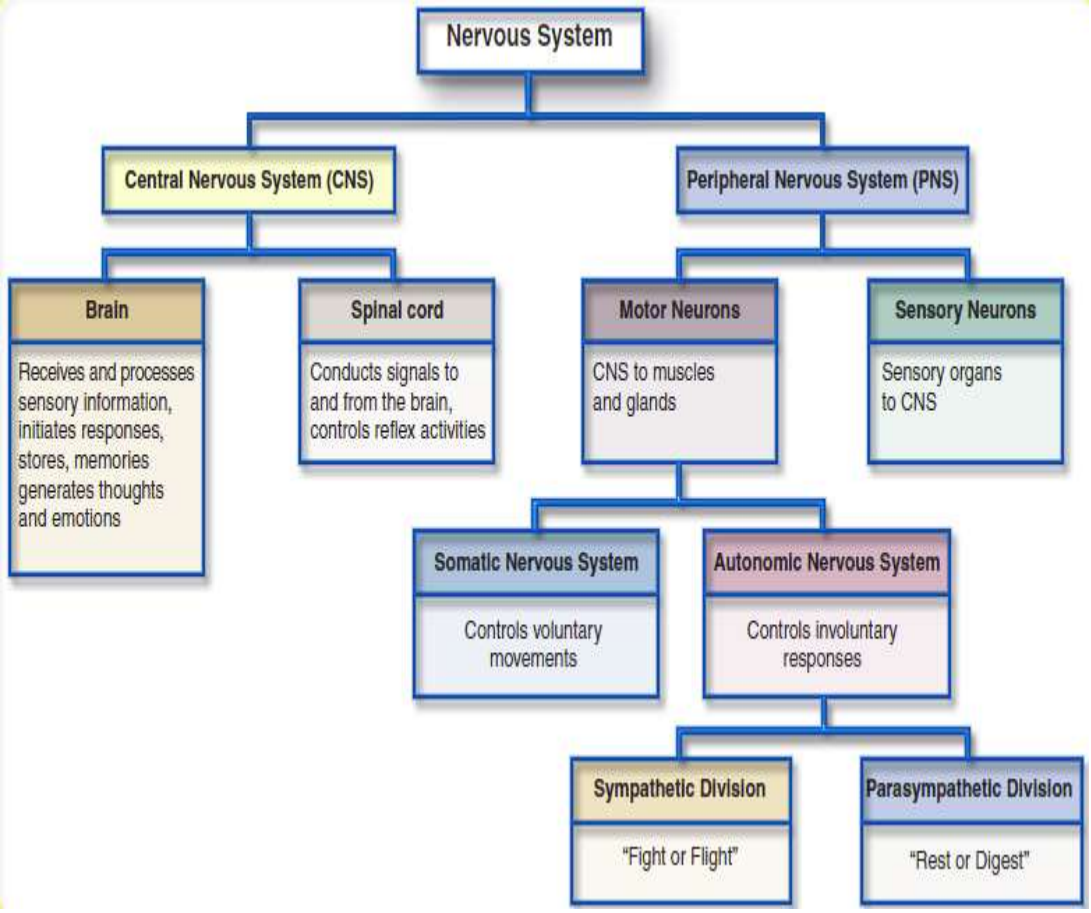


Figure-3: Organization of the Human Nervous System.

1.3.2.Thecentral Nervous System:

The "Central Nervous System", comprised of brain, brainstem, and spinal cord. The central nervous system (CNS) represents the largest part of the nervous system, including the brain and the spinal cord. Together, with the peripheral nervous system (PNS), it has a fundamental role in the control of behavior. The CNS is conceived as a

system devoted to information processing, where an appropriate motor output is computed as a response to a sensory input. CNS is protected by Bone (skull, vertebrae). They are also wrapped up in three protective membranes called meninges (spinal meningitis is infection of these membranes). Spaces between meninges filled with cerebrospinal fluid for cushioning and protection. This fluid also found within central canal of the spinal cord and ventricle of brain.

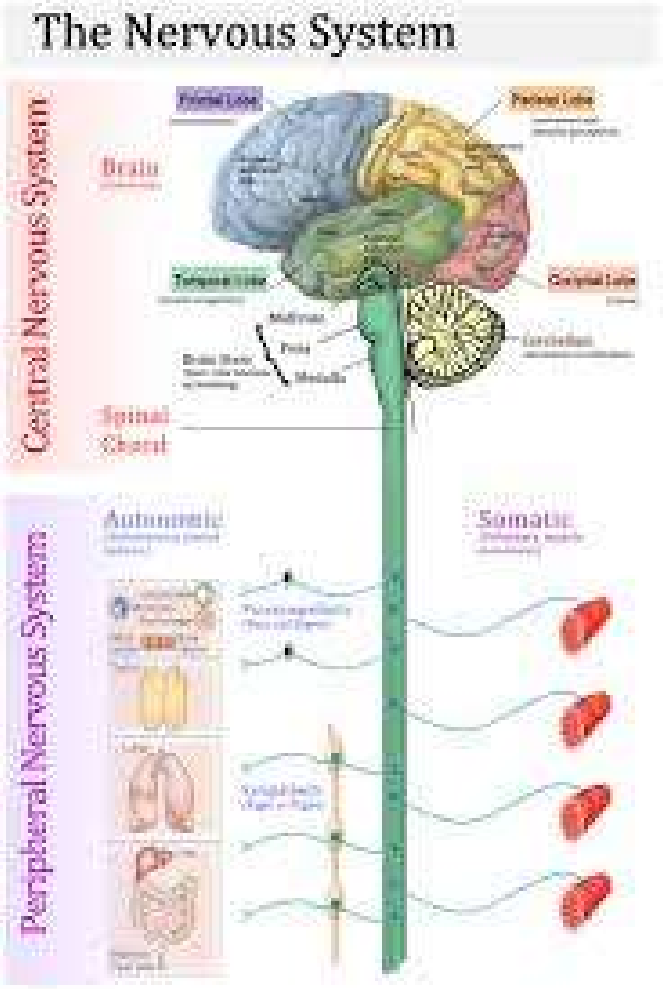


Figure-4: The Nervous System

1.3.3.Brain:

The brain itself contains parts which function in the coordination of movement, sensing and consciousness (and all that entails), as well as areas that are below the level of

conscious control. The brain has a volume,on average, or 1,370 cubic centimeters (with a normal range of 950 to 2,200 cm2). It weighs about 1.35 kg (or 3 pounds), and consists of hundreds of billions of neurons and glial cells.The brain is vastly complex, and is certainly notthoroughly understood. There are many ways of looking at the brain functionally and structurally.

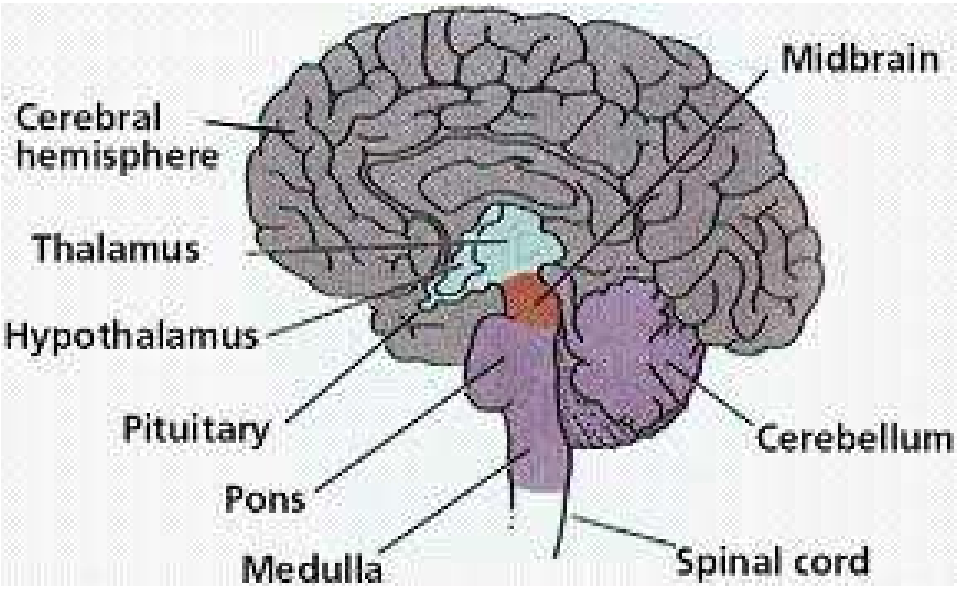


Figure-5: Human Brain

Medulla:

The medulla is the control center for respiratory, cardiovascular and digestive functions.

Pons:

The pons houses the control centers for respiration and inhibitory functions. Here it will interact with the cerebellum.

Cerebrum:

The cerebrum, or top portion of the brain, is divided by a deep crevice, called the longitudinal sulcus. The longitudinal sulcus separates the cerebrum in to the right and left hemispheres. In the hemispheres you will find the cerebral cortex, basal ganglia and

the limbic system. The two hemispheres are connected by a bundle of nerve fibers called the corpus callosum.

The right hemisphere is responsible for the left side of the body while the opposite is true of the left hemisphere. Each of the two hemispheres are divided into four separated lobes: the frontal in control of specialized motor control, learning, planning and speech; parietal in control of somatic sensory functions; occipital in control of vision; and temporal lobes which consists of hearing centers and some speech. Located deep to the temporal lobe of the cerebrum is the insula.

Cerebellum:

The cerebellum is the part of the brain that is located posterior to the medulla oblongata and pons. It coordinates skeletal muscles to produce smooth, graceful motions. The cerebellum receives information from our eyes, ears, muscles, and joints about what position our body is currently in (proprioception). It also receives output from the cerebral cortex about where these parts should be. After processing this information, the cerebellum sends motor impulses from the brainstem to the skeletal muscles. The main function of the cerebellum is coordination. The cerebellum is also responsible for balance and posture. It also assists us when we are learning a new motor skill, such as playing a sport or musical instrument. Recent research shows that apart from motor functions cerebellum also has some emotional role.

Spinal Cord:

The spinal cord extends from the foramen magnum at the base of the skull to the level of the first lumbar vertebra. The cord is continuous with the medulla oblongata at the foramen magnum. Like the brain, the spinal cord is surrounded by bone, meninges, and cerebrospinal fluid. The spinal cord is divided into 31 segments with each segment giving rise to a pair of spinal nerves.

At the distal end of the cord, many spinal nerves extend beyond the conusmedullaris to form a collection that resembles a horse's tail. This is the caudaequina. In cross section, the spinal cord appears oval in shape

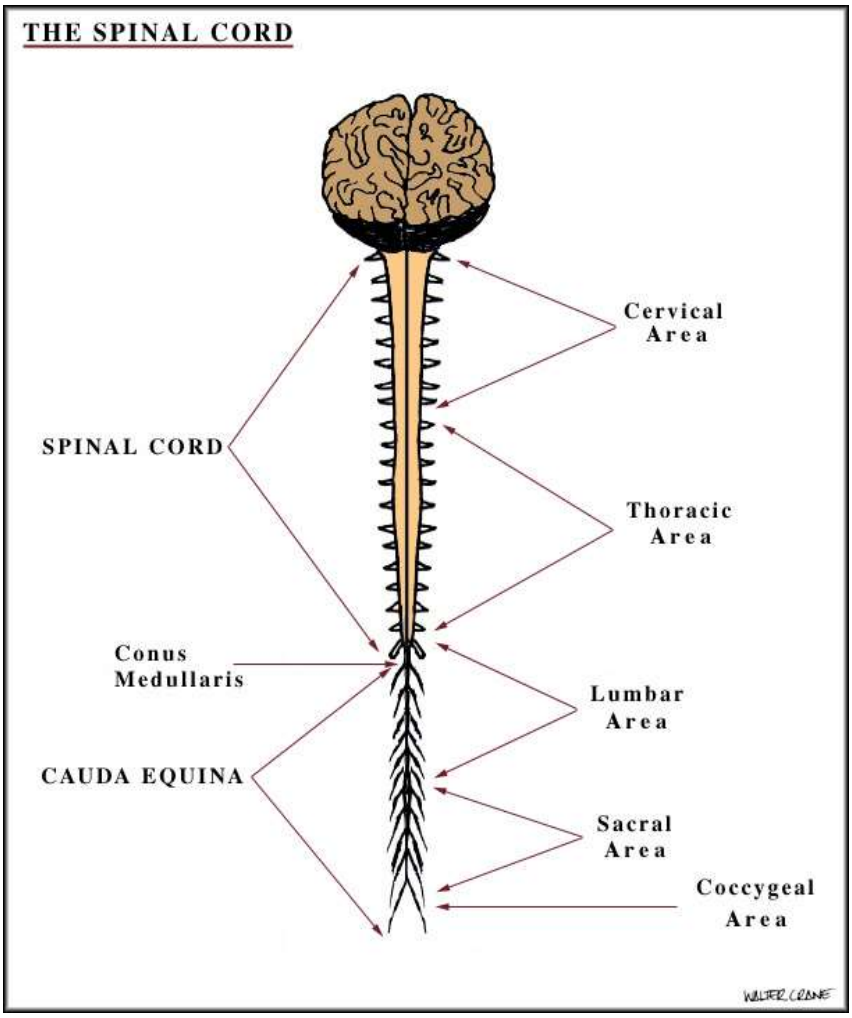


Figure-6: The Spinal cord.

Spinal cord contains central canal filled with cerebrospinal fluid,Gray matter and white matter. Gray matter (inner layer) containing cell bodies of neurons and short fibers and lookslike a butterfly with open wings. in grey matter, dorsal cell bodies functionprimarily in receiving sensory information, and ventral cell bodiessend along primarily motor information.

White matter (outer layer) containing long fibers of interneurons that run together in bundles called tracts that connect the cord to the brain. within white matter, ascending tracts take information to the brain, descending tracts in the ventral partcarry information down from the brain.(Kandel, 2000)

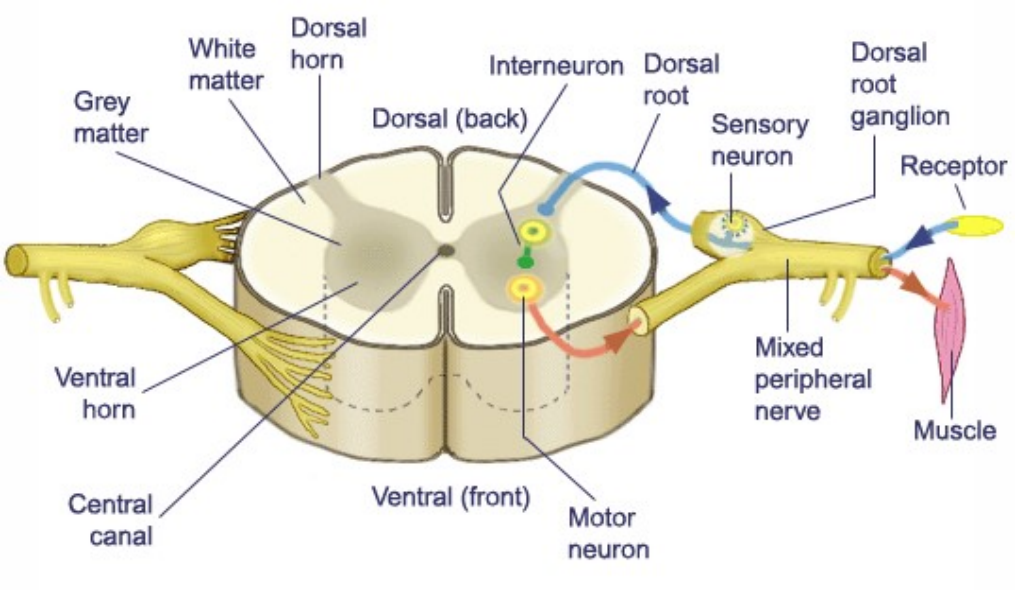


Figure-7: Cross section of spinal cord.

1.3.4 Peripheral Nervous System:

The peripheral nervous system includes nerves that carry sensorymessages to the central nervous systemand nerves that send information fromthe CNS to the muscles and glands.The peripheral nervous system isfurther divided into the somatic systemand the autonomic system. The peripheral nervous system includes 12 cranial nerves 31 pairs of spinal nerves.

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

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

Autonomic Nervous System: The autonomic system controls glandular secretions and the functioning of the smooth and cardiac muscles. The sympathetic and parasympathetic divisions of the autonomic system oftenwork in opposition to each

other toregulate the involuntary processes ofthe body. Involuntary processes, such as heartbeat and peristalsis, are those that donot require or involve conscious control. (wikibooks , 2014)

1.3.5 Nervous Tissue:

The nervous system coordinates the activity of the muscles, monitors the organs, constructs and also stops input from the senses, and initiates actions. Prominent participants in a nervous system include neurons and nerves, which play roles in such coordination. Our nervous tissue only consists of two types of cells. These cells are neurons and neuroglia cells. The neurons are responsible for transmitting nerve impulses. Neuroglia cells are responsible for supporting and nourishing the neuron cells.

1.3.6 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.

Cell Body or Soma:

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

Axon:

Cell body gives rise to a tubular process which is the main conducting unit of the neuron, capable of conveying information at great distances by propagating transient

electrical signal called action potential. Many axons are surrounded by a segmented, white, fatty substance called myelin or the myelin sheath. Myelinated fibers make up the white matter in the CNS, while cell bodies and unmyelinated fibers make the gray matter. The unmyelinated regions between the myelin segments are called the nodes of ranvier. Thus, axons are of two types, myelinated and non-myelinated.

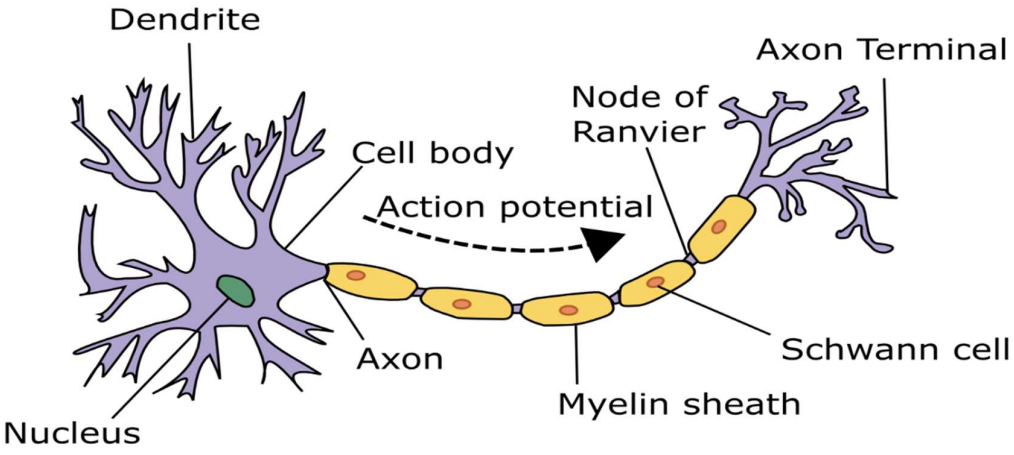


Figure-8 : Neuron

Dendrites:

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

1.3.7Types of neurons:

- **Sensory neuron** (afferent neuron) : It takes a message from a sense organ to CNS. has long dendrite and short axon.
- **Motor neuron** (efferent neuron) :It takes message away from CNS to amuscle fiber or gland. Short dendrites,long axon.

- **Interneuron** (association neuron or connector neuron): completely contained within CNS. Conveys messages between parts of the system. Dendrites, axons, may be long or short.(Martini, 2003)

1.3.8Synapse:

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.

1.3.9Different Types of Synapse:

The human nervous system uses a number of different neurotransmitter and neuroreceptors, and they don't all work in the same way. We can group synapses into 5 types:

1. **Excitatory Ion Channel Synapses.**
   These synapses have neuroreceptors that are sodium channels. When the channels open, positive ions flow in, causing a local depolarisation and making an action potential more likely. Typical neurotransmitters are acetylcholine, glutamate or aspartate.
2. **Inhibitory Ion Channel Synapses.**
   These synapses have neuroreceptors that are chloride channels. When the channels open, negative ions flow in causing a local hyperpolarisation and making an action potential less likely. So with these synapses an impulse in one neurone can inhibit an impulse in the next. Typical neurotransmitters are glycine or GABA.
3. **Non Channel Synapses.**
   These synapses have neuroreceptors that are not channels at all, but instead are membrane bound enzymes. When activated by the neurotransmitter, they catalyse the production of a "messenger chemical" inside the cell, which in turn can affect many aspects of the cell's metabolism. In particular they can alter the number and sensitivity of the ion channel receptors in the same cell. These synapses are involved in slow and long-lasting responses like learning and memory. Typical

neurotransmitters are adrenaline, noradrenaline (NB adrenaline is called epinephrine in America), dopamine, serotonin, endorphin, angiotensin, and acetylcholine.

4. **Neuromuscular Junctions.**

These are the synapses formed between motor neurones and muscle cells. They always use the neurotransmitter acetylcholine and are always excitatory. We shall look at these when we do muscles. Motor neurones also form specialised synapses with secretory cells.

5. **Electrical Synapses:**

In these synapses the membranes of the two cells actually touch, and they share proteins. This allows the action potential to pass directly from one membrane to the next. They are very fast, but are quite rare, found only in the heart and the eye.

1.3.10Neurohormones :

Peptide-secreting cells of the hypothalamicohypophyseal circuits originally were described as neurosecretory cells, receiving synaptic information from other central secreted into the blood by a neuron. This term has lost much of its original meaning, because these neurons, yet secreting transmitters in a hormone-like fashion into the circulation. The trans neurons also may form traditional synapses with central neurons. Cytochemical evidence indicates that the same substances that are secreted as hormones from the posterior mitter released from such neurons was termed a *neurohormone*, *i.e.,* a substance hypothalamic pituitary (oxytocin, arginine-vasopressin) mediate transmission at these sites. Thus the designation *hormone* relates to the site of release at the posterior pituitary and does not necessarily describe all the actions of the peptide.

1.3.11Neurotransmitters:

Nerve impulse is carried by chemicals called neurotransmitters. These chemicals are made by the cell that is sending the impulse (the pre-synaptic neurone) and stored in synaptic vesicles at the end of the axon. The cell that is receiving the nerve impulse

(the post-synaptic neurone) has chemical-gated ion channels in its membrane, called neuroreceptors. These have specific binding sites for the neurotransmitter**.**

Table-3: List of Neurotransmitters with Their Sites

Group	**Neurotransmitter**	**Region of Operation**
Acetylcholine	Acetylcholine	Central Nervous System (CNS), Peripheral Nervous System (PNS) and Autonomic Nervous System (ANS)
Serotonin	Serotonin	CNS and PNS
Amino acids	Glutamate, Gamma Aminobutyric Acid (GABA), Glycine, Aspartate	CNS
Histamine	Histamine	Hypothalamus
Catecholamines	Norepinephrine, Epinephrine (Adrenalin)	CNS and Sympathetic Nervous System
Neuropeptides	Endorphins (Enkephalins and Dynorphins)	CNS
Dopamine	Dopamine	CNS
Nucleotides	Adenosine, Adenosine Triphosphate (ATP)	CNS PNS and ANS
Nitric oxide	Nitric oxide	CNS

1.3.12 Functions of Neurotransmitters:

The neurotransmitters are stored in tiny sac-like structures called vesicles at the end of axons. When an impulse, or nerve signal, reaches the end of the axon, the vesicles release neurotransmitters into the small space between the adjoining cells known as synaptic gap. Neurotransmitters diffuse across the synapse and bind to receptors in the receiving cell that are specific for the neurotransmitter.

There are several types of neurotransmitter each contributing in specific and important functions of central nervous system. Here is a glance of the functions done by some important neurotransmitters:

Acetylcholine: It is involved in learning and memory. It also slows the heart, makes the bronchi and gut contract, stimulates glands to produce saliva and mucus.

Dopamine: It controls movement and regulates the flow of information coming into the brain.

Norepinephrine: It isinvolved in the regulation of mood**.** It causes the heart rate and blood pressure to increase. It also increases the conversion of glycogen to glucose in the liver, increases the conversion of fats to fatty acids in fat tissue, and it relaxes bronchial smooth muscle to open up air passages to the lungs. It is also important for learning and forming memories.

Oxytocin: It plays a large part in establishing maternal behavior. It also induces labor and is associated with well-being in relationships.

Phenyl ethylamine: It causes feelings of happiness and relieves depression. It also increases attention and activity, promotes energy, elevates mood, and favors aggression.

Serotonin: Like norepinephrine, it regulates mood, emotion, sleep, and appetite. Decreased serotonin contributes to depression.

GABA: GABA reduces physical tension, anxiety, insomnia, blood pressure. It elevates pain threshold and decreases heart rate.

Prolactin: The functions of prolactin are inhibition of dopamine. It decreases sex hormones like estrogen in women and testosterone in men.

Nitric oxide: Nitric oxide does vasodilatation and it thins blood. It reduces platelet stickiness and blood coagulation.

Neuromodulators:

The distinctive feature of a modulator is that it originates from nonsynaptic sites, yet influences the excitability of nerve cells. Florey (1967) specifically designated substances such as CO_2 and ammonia, arising from active neurons or glia, as potential modulators through nonsynaptic actions. Similarly, circulating steroid hormones, steroids produced in the nervous system (*i.e.*, neurosteroids), locally released adenosine, and other purines, eicosanoids, and nitric oxide (NO) are all now regarded as modulators.

1.3.13Neuromediators:

Substances that participate in eliciting the postsynaptic response to a transmitter fall under this heading. The clearest examples of such effects are provided by the involvement of cyclic AMP, cyclic GMP, and inositol phosphates as second messengers at specific sites of synaptic transmission. However, it is technically difficult to demonstrate in brain that a change in the concentration of cyclic nucleotides occurs prior to the generation of the synaptic potential and that this change in concentration is both necessary and sufficient for its generation. It is possible that changes in the

concentration of second messengers can occur and enhance the generation of synaptic potentials. Second messenger-dependent protein phosphorylation can initiate a complex cascade of molecular events that regulate the properties of membrane and cytoplasmic proteins central to neuronal excitability (Gyton, 2006) . These possibilities are particularly pertinent to the action of drugs that augment or reduce transmitter effects.

1.3.14 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 clot (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 isnot 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).

1.4.1Toxicity

The side effects of synthetic chemical drugs have emerged in today's society, thus there is growing interest in natural products and scientific studies are actively being conducted on traditional herbal medicines.(Shin et al., 2013).Ethnomedicinal plants are important resources of natural products. Most ethnomedicinal plants are greatly lack of systematic safety evaluation using modern technology due to the academic and historical reasons (B.-G. Ye et al 2014)

1.4.2Toxic Herbal Medicines

Herbs that contain carcinogens

Comfrey *(Symphytum officinale)* -
Coltsfoot *(Tussilago farfara)* -
Borage *(Borago officinalis)* -
Life root *(Senecio aureus)* -
Sassafras *(Sassafras albidum)* -
Calamus *(Acorus calamus)* -
Chinese herbs containing aristolochic acid**, e.g.,** GuanMuTong*(Aristolochia fangchi, Aristolochia manshuriensis)*

1.4.3 Herbs that cause cardiotoxicity and respiratory depression

Ephedra *(Ephedra sinica)* -
Pokeroot *(Phytolacca americana)* -
Senna *(Cassia acutifolia)* -
Lobelia *(Lobelia inflata)* -
Oleander *(Nerium oleander)*

1.4.4 Herbs that cause CNS toxicity

Ephedra *(Ephedra sinica)*

Yohimbine *(Corynanthe yohimbe)*

Camphor *(Cinnamomum camphorum*

Table 4: list of herbal product contradiction and drug interaction

CONTRADINDICATION AND/OR DRUG INTERACTION	HERBAL PRODUCT
Pregnancy and/or Lactation	Black Cohosh Chamomile Dong Quai Feverfew Ginseng Kava Licorice St. John's Wort
Gallstones	Ginger
Autoimmune Disorders or use of Immunosuppressive Drug or Potentially Hepatotoxic Drugs	Echinacea
Warfarin	Dong Quai Feverfew Garlic Ginkgo Ginseng Danshen
Hypertension, CHF and/or use of Digoxin, Diuretics and other Heart Medications	Ginseng Hawthorn Licorice Ephedrine-containing Senna Pokeroot
Estrogenic or Androgenic Therapies or Anti-therapies	Black Cohosh Dong Quai Saw Palmetto
CNS Disorders or use of CNS Medications	Ephedrine-containing Evening Primrose Ginkgo Ginseng Kava St. John's Wort Valerian Y ohimbine

1.4.5Causes of toxicity with herbal products

All chemicals may be considered toxic under certain conditions, e.g. even pure waterwhen inhaled is rapidly absorbed across the lung alveoli to cause lysis of red blood cells.But some chemicals present a greater hazard than others (Pascoe, 1983). A

large number of plants contain appreciable levels of biosynthetically produced chemical substances and many of these have either been reported to be toxic to humans or are predictably toxic based on extensive animal or *in vitro* studies (Tomlinson and Akerele, 1998).

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

• In the first category, as previously mentioned, the toxicity may be as a result of exposure to intrinsic ingredients of some medicinal plants. Examples of some more important classes of ingredients implicated here include: p*yrrolizidinealkaloids,* which are said to be hepatocarcinogens; a*ristolochic acid I*, said to bemutagenic and carcinogenic; *phorbol esters,* which are tumor promoters andvesicant to the skin; *carboxyactractyloside*, a deadly toxic compound; a*mygdalin*,a cyanogenic compound with many undesired effects; etc. (Gaillard and Pepin,1999; Tomlinson and Akerele, 1998). In addition, several studies conducted on flavonoids indicate that, besides their apparently beneficial health effects, they may also induce mutagenicity and genotoxicity (e.g. quercetin) in both bacterialand mammalian experimental systems (Skibola and Smith, 2000).

• The second category of causes of toxicity of herbal medicines is more extrinsic or non-associated with the plant active constituents. In this category, the toxicity is a result of exposure to plant products contaminated with excessive or banned pesticides, microbial contaminants, heavy metals or chemical toxins, or with substituted ingredients. The pesticide, heavy metal and microbial contaminants may be linked to the source, collection or processing of the herbal materials (e.g.in contaminated environments). Chemical toxins may arise due to incorrect storage conditions or chemical treatment during storage (Chan, 2003; Gaillard and Pepin, 1999; Tomlinson and Akerele, 1998). Some of these environmental factors can be controlled by implementing standard operating procedures that lead to good agricultural, good laboratory, good supply and good manufacturing practices for producing medicinal products from herbal or natural sources (Chan, 2003).

Given the above, what then is the current prevalence of toxicity with herbal products.

1.4.6Prevalence of toxicity with herbal products

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

From published reports, it appears that side effects or toxic reactions, of any form but associated with herbal medicines, are rare (Tomlinson and Akerele, 1998). This may be because herbal medicines are generally safe, that adverse reactions following their use are underreported, or because the side effects are of such a nature that they are not reported (Tomlinson and Akerele, 1998; Gaillard and Pepin, 1999).

There is, therefore, a need for the public to have an understanding of the risks posed by herbal medicines so as to ensure that such products are used safely. Particularly, as highlighted above, the safety of some herbal products is compromised by lack of suitable control of the quality of herbal medicines and the absence of appropriate herbal use information for patients. To appropriately inform and protect the public, the herbal products must, however, first be evaluated for its toxicity.

1.4.7 Evaluation of herbal toxicity

Herbal toxicity can be evaluated by (1) observing human or animal populations exposed to the plant material, (2) administering the plant medicine to animals under controlled conditions and observing the effects *(in vivo)* and (3) exposing cells, sub-cellularfractions or single-celled organisms to the plant material *(in vitro)*(Timbrell, 2002). Inthis report the focus is more on the *in vivo* model.

Ethically, toxicity of a compound cannot randomly be evaluated in humans. Nevertheless, the exposure of humans to toxicity with herbal materials, as highlighted previously on point 2.2.2.1, may occur accidentally when these are part of their therapeutic activity intentionally as with drugs and food additives (Chan, 2003). In such cases, the accidents resulting from this type of exposure may, if well monitored and recorded (i.e.by measuring substances and their metabolites in body fluids and using biological in dices of pathological change), provide important information about the toxicity of a plant material in humans. However, acquiring such data is often difficult and rarely complete, and the latter is the main reason why procedures for animal toxicity testing have been maintained as a successful alternative for evaluating the harmfulness of compounds for humans (Timbrell, 2002; Tomlinson and Akerele, 1998).

As previously reported in sections 2.1.2 and 2.1.3, to date, the majority of data on the toxicity of chemicals, including drugs and herbal medicines, is gained from experimental studies done in animals (*in vivo*). The data so acquired are used for the risk assessment and safety evaluation of drugs (or herbal medicines) prior to human exposure. Because animal tests can be carefully controlled with the exact known doses being used, the quality of the data obtained is generally reliable (Timbrell, 2002). The number of animals used should be enough to allow statistical significance to be demonstrated and the application of humane conditions and proper treatment of the animals are essential, for scientific as well as ethical reasons, to help ensure that the data are reliable and robust(Timbrell, 2002; Chan, 2003).

In a toxicity study, the animal species selected will depend partly on the type of toxicity test, existing data available and also on ethical and financial considerations. The most common species used are rats and mice for reasons of size, accumulated knowledge on these species and cost, besides the similarity of their metabolism to that of humans (Timbrell, 2002; Loomis and Hayes, 1996).

In the present study, the concern was on the evaluation of the toxicity of Sp.acmella the administration of the plant material to rodents. As part of the effort of quality assurance of herbal products, scientific experiments were to be carried out to assess the safety of this medicinal plant using the rodent as an alternative mammalian species.

1.4.8Exposure

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.

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)

1.4.9Definition of toxicity

Toxicity is defined as “the potential of a substance to exert a harmful effect on humans or animals, and a description of the effect and the conditions or concentration under which the effect takes place” (Health and safety,2004). In order to support an application for a clinical trial or for the registration of a new drug, it is necessary to satisfy legislation that requires that certain data should be produced from a variety of toxicological investigations that show the safety profile of the compound to which humans may be exposed. Therefore, in the majority of cases of evaluation of the toxicity of most substances, rodents and non-human primates are first used in preclinical animal safety studies before further studies are done in humans. These animals are mainly used because of their biological similarity to humans that allows them to be regarded as the suitable metabolic models for humans in a broad range of investigations (Loomis and Hayes, 1996; Pascoe, 1983). In general, toxicity testing methods can be divided into two categories: The first category comprises tests that are designed to evaluate the overall effects of compounds on experimental animals. Individual tests in this category differ from each other basically in regard to the

duration of the test and the extent to which the animals are evaluated for general toxicity. These tests are classified as acute, prolonged and chronic toxicity tests (Loomis and Hayes, 1996). The second category of tests consists of those that are designed to evaluate specific types of toxicity in detail. The prolonged and chronic tests do not detect all forms of toxicity, but they may reveal some of the specific toxicities and indicate the need for more detailed studies. Thus, this second category of tests has been developed for the determination of effects of compounds on the fetus in a pregnant animal (teratogenic tests), on the reproductive capacity of the animals (reproduction hand, chronic effects are often detected over an extended period of time during which exposure may be continuous or intermittent, though obviously at levels which are too low to produce an acute effect (Loomis and Hayes, 1996; Pascoe, 1983).

1.4.10 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; or any poisonous effect resulting from a single short-term exposure to a toxic substance" (Association of Vermont Recyclers, 1996).

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; Pascoe, 1983).

Furthermore, acute toxicity tests are those designed to determine the effects, which occur within a short period after dosing. They serve to establish the lethal dose range of the test substance and provide prompt warning if a highly toxic compound is being dealt with (Poole and Leslie, 1989). They also provide information on the limiting toxicity arising from the pharmacological effects of the compound on target organs and, often, on the maximum dose to be used in subsequent sub-acute studies (chronic studies). This latter information is particularly important for predicting the amount of chemical required for future toxicological studies (Poole and Leslie, 1989).

The initial procedure, in an acute toxicity test programme, is to test a series of rangefinding single doses of the compound in a single animal species. This necessitates selection of a route of administration, preparation of the compound in a form suitable for administration via the selected route and selection of an appropriate experimental animalspecies (Poole and Leslie, 1989). Generally, even if the intended use of the compound does not involve the oral or parenteral routes, at least the oral route is used, in addition to other routes, so that comparison with other related compounds can be made. Additionally, such testing may also indicate the viability of the oral route for use in subsequent, more extensive and prolonged toxicity studies (Loomis and Hayes, 1996). Normally, the significance and use of the data that are obtained are limited to those routes of administration that were used in the actual experiment.

As usual, all initial acute toxicity tests are performed on either rats or mice because of the low cost, the availability of the animals, and the fact that abundant reference toxicologic data for many compounds in these species are available (Loomis and Hayes, 1996). In addition, these animals generally metabolize compounds in a similar manner to humans and the compounds (including metabolites) may have similar pharmacodynamics in the animals and humans. Before the experiment is performed, a total number of animals of similar body weight and same sex, or equal numbers of both sexes, are selected and randomly assigned to test (treatment) and control groups. After exposure to single doses of the test compound (or treatment), the animals are monitored for a minimum of 24hrsfor any clearly recognized effect (such as changes in locomotor activity; bizarre reactions; sensitivity to pain, sound and touch; changes in social interaction; aggressive behavior; convulsions; paralysis, etc.) seen, as an index of toxicity, shortly or/and consistently after the administration of the chemical (Timbrell, 2002; Loomis and Hayes). However, the most easily recognized and certainly the most significant of effects is that of death and this outcome is usually used as a primary measure of acute toxicity. If the animals appear to be healthy at the end of 24hrs, they are monitored at daily intervals for at least a further one to two weeks for the appearance of delayed toxicity (Loomis and Hayes, 1996; Pascoe, 1983; Timbrell, 2002). In the rodents, three types of acute toxicity studies may be performed. For the first type of study, it is usual to establish the maximum tolerated dose (i.e. the highest dose after which the animals recover completely from all effects of the chemical) and the minimum lethal dose for the compound (or treatment). The second type of study is

the single dose study to establish the target organ(s) for toxicity while the third type of study is for the determination of the precise LD50 or median lethal dose. The results of the latter type of study may be required, in most countries, for a clinical trial's certificate or even for a product license (Poole and Leslie, 1989; Timbrell, 2002; Pascoe, 1983). Usually, to establish a LD50, at least four dose levels are used, with 5-10 male animals plus 5-10 females per treatment group. The animals are given a single dose of test compound and, at the end of 14-days observation period, the major organs and abnormal tissues of the surviving animals are collected and subjected to histopathological investigation. The LD50 and its confidence limits are calculated from the lethality data using probit analysis (Pascoe, 1983). Since a great range of concentrations or doses of various chemicals may be involved in the production of harmful effects, the LD50 has been used by some authors to devise categories of toxicity on the basis of the amounts of the chemicals necessary to produce harm. An example of such a categorization, along with the respective lethal doses, is given in the following Table

Table 5: Classification of toxicity based on LD^{50} dose ranges

Category	LD50 (mg/kg)	LD50 (mg/kg)	Classification
Extremely toxic	1 or less	< 5	Super-toxic
Highly toxic	1 to 50	5- 50	Extremely toxic
Moderately toxic	50 to 500	50- 500	Very toxic
Slightly toxic	500 to 5000	500- 5000	Moderately toxic
Practically non-toxic	5000 to 15000	5000- 15000	Slightly toxic
Relatively harmless	More than 15000	> 15000	Practically non-toxic
(Loomis and Hayes, 1996)		(Pascoe, 1983)	

Acute exposure is defined as exposure to a chemical for less than 24 h. Acuteexposure by inhalation refers to continuous exposure for less than 24 h, most frequently for 4 h. Subacute exposure refers to repeated exposure to a chemical for 1 month or less.

1.4.11Chronic 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 or over a significant fraction of an animal's or human's lifetime "(Association of Vermont Recyclers, 1996).

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 (Poole and Leslie, 1989). The dose levels of compounds used usually range from a very low fraction of the therapeutically effective dose (i.e. somewhere in the range of the ED50 for the compound in that species, or of the same order as the anticipated human therapeutic dose range) to doses that approach the maximum non-lethal dose (as established in rodent acute toxicity studies) (Poole and Leslie, 1989; Loomis and Hayes, 1996).

Different approaches to dose ranging studies are applied depending on the species being used. The procedures used can vary, but usually involve exposing the experimental animals (in typical group sizes of two to five animals/sex/group) to various doses of the test compound, i.e. from the maximal non-lethal dose (determined in the acute studies) down to doses in the pharmacological dose range. Clinical chemistry and haematological parameters are then measured at the start of the study (i.e. within 48 hours after the first dose) and at the end of the study, along with full histopathology analysis of all abnormal tissues plus the major organs (such as the heart, liver, kidneys, lungs and brain of the animals), at least at the end of the study (Poole and Leslie, 1989; Timbrell, 2002).

- ***Subchronic* for 1 to 3 months**
- ***Chronic* for more than 3 months**.

1.4.12 Toxic effects

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

In all the cases, the toxic effects are usually manifested either in an acute or a chronic manner, and occur mostly as a result of an acute or chronic exposure to toxic compound by oral ingestion, inhalation or absorption following skin contact the toxic effects are seen as (1) signs or reflection of a disturbance of the normal activities of enzymes that perform essential biochemical roles in all forms of life; (2) alteration of the normal activities of plasma membrane that regulate the exchange of nutrients and metabolites between the cell and its surroundings and (3) the disturbances of other normal cell activities, e.g. RNA and DNA synthesis, growth, division and general metabolism at all levels of organization from sub-cellular to organ and organ system (Pascoe, 1983; Timbrell, 2002).

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

1.4.13Routes of administration

This term refers to the way in which drugs or compounds are introduced to animal's orhumans. To evaluate toxicity of a compound in animals various routes may be used, but two most commonly used modes of administration for animals studies are via intra-peritoneal injection or the oral route (Poole and Leslie, 1989).

Intra-peritoneal injection

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

Oral administration

The oral route is probably one of the most common means by which a chemical enters the body. In short, the oral administration is the form of administration involving the gastrointestinal tract, which may be viewed as a tube going through the body, starting at the mouth and ending at the anus. Although it is within the body, its contents are essentially exterior to the body fluids. Most orally administered chemicals can otherwise have a systemic effect on the organism only after absorption has occurred from the mouth or the gastrointestinal tract. Oral administration of chemicals that are rapidly absorbed from the gastrointestinal tract would theoretically expose the liver to concentrations of the agent that would not be obtained if other routes of administration were used (Loomis and Hayes, 1996). Furthermore, if a compound entered the enterohepatic cycle, at least a portion of the compound would be localized in the organs involved in the cycle .Compounds that are known to be toxic to the liver would be expected to be more toxic following oral administration on repeated occasions, whereas their administration by other routes may be less hazardous (Loomis and Hayes, 1996; Waynforth, 1980).

1.5 Hematology

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

1.5.1 History of Cell counting

Leeuwenhoek was the first person who attempted to count blood cells using a glass capillary tube with graduation marks of measured dimension and microscope to count. He selected chicken to count red blood cells [Hajdu, SI 1998;42:1075]. Afterwards, different techniques were introduced for diluting the blood which resulted in more accurate and easier counting using a shallow rectangular chamber which had a thin cover glass and diluted blood was injected into this glass. In the early 20th century a technique using photoelectric device to count cells was invented by Moldovan

[Bennett, 1841.] However, this attempt for cell counting did not develop at that time because of the unreliability of the photoelectric device. An automated blood-cell counter technique was invented by Waiter H. Coulter [Hajdu, SI 1998;42:1075] in the mid 1950's for blood cell counting. The research was based on the technique known as "Coulter's Principle" or the Aperture Impedance technique. This technique uses the resistivity of the blood cells because thee impedance of the cells suspended in the diluting fluid is much more higher than that of fluid was based on the fact that the resistivity of blood cells is much higher than that of the diluting fluid. Most modern cell counters serves on the basis of this extensively developed since 1950's.

1.5.2 Cellular Elements of Blood

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

Blood is made of two parts:

1. Plasma which makes up 55% of blood volume.
2. Formed cellular elements (red and white blood cells, and platelets) which combine to make the remaining 45% of blood volume (Alberts, 2012).

1.5.3 Plasma

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

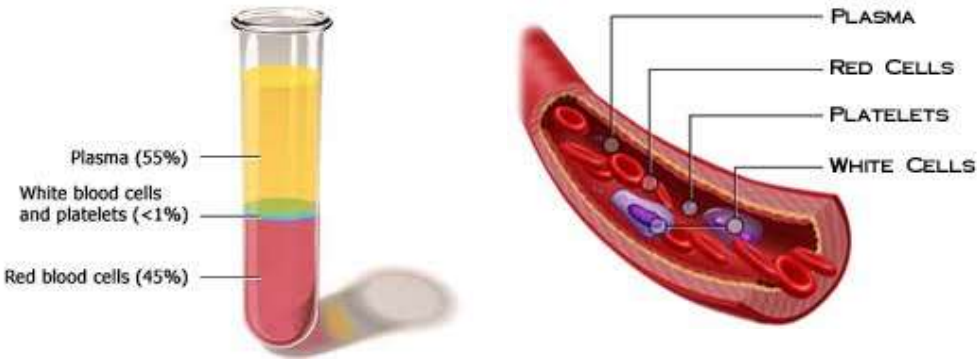


Figure-9: Plasma of the Blood

1.5.4 Cellular Elements

1.5.4.1 Red Blood Cell

RBCS have a shape of a disk that appears to be “caved in” or almost flattened in the middle; this is called bi-concave. This bi-concave shape allows the RBC to carry oxygen and pass through even the smallest capillaries in the lungs. This shape also allows RBCs to stack like dinner plates and bend as they flow smoothly through the narrow blood vessels in the body. RBCs lack a nucleus (no DNA) and no organelles, meaning that these cells cannot divide or replicate themselves like the cells in our skin and muscles. RBCs have a short life span of about 120 days, however, as long as our myeloid tissue is working correctly, we will produce about 2-3 million RBCs per second. That is about 200 billion a day! This allows us to have more to replace the ones we lose. The main component of the RBC is hemoglobin protein, of which there are about 250 million per cell. The word hemoglobin comes from "hemo" meaning blood and "globin" meaning protein. Hemoglobin is composed of four protein subunits: polypeptide globin chains that contain anywhere from 141 to 146 amino acids. Hemoglobin is responsible for the cell’s ability to transport oxygen and carbon dioxide. Normal range of RBC 8-16×106mm3(Robert B, 2006).

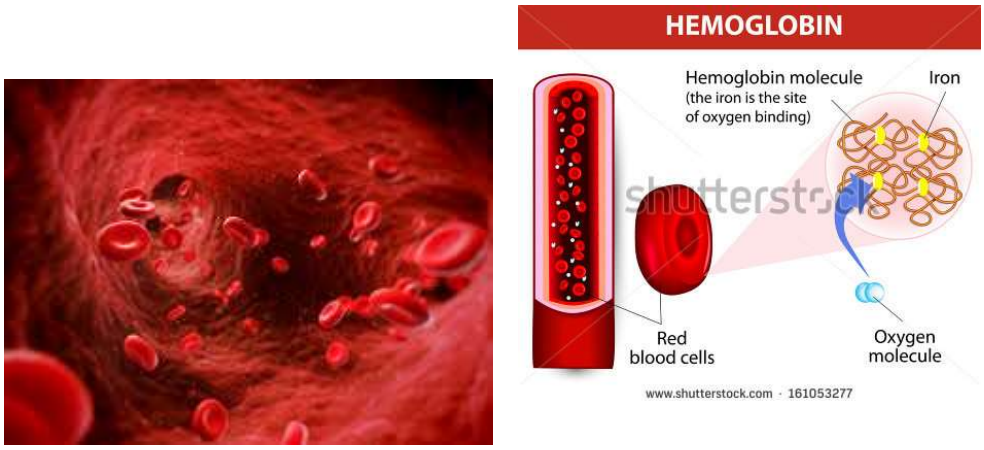


Figure-10: Red Blood Cell & Hemoglobin

1.5.4.1Different count of RBC

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

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

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

Normal results for adults vary, but in general are:

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

Lower than Normal Hemoglobin

Low hemoglobin level may be due to:

- Anemia due to red blood cells being destroyed earlier than normal (hemolytic anemia)
- Anemia (various types)
- Bleeding from digestive tract or bladder, heavy menstrual periods
- Chronic kidney disease
- Bone marrow being unable to produce new blood cells. This may be due to leukemia, other cancers, drug toxicity, radiation therapy, infection, or bone marrow disorders
- Poor nutrition
- Low level of iron, folate, vitamin B12, or vitamin B6
- Other chronic illness, such as rheumatoid arthritis

Higher than Normal Hemoglobin

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

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

ii)Hematocrit (HCT)

The hematocrit (Ht or HCT, British English spelling haematocrit), also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood cells in blood. It is normally 45% for men and 40% for women. It is considered an integral part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count, and platelet count. Anemia refers to an abnormally low hematocrit, as opposed to polycythemia, which refers to an abnormally high hematocrit. Both are potentially life-threatening disorders (Purves, 2004).

Higher than Normal Hematocrit

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

Lower than Normal Hematocrit

- Infants without adequate iron intake
- children going through a rapid growth spurt, during which the iron available cannot keep up with the demands for a growing red cell mass

- menstruating women, who have a greater need for iron because of blood loss during menstruation
- pregnant women, in whom the growing fetus creates a high demand for iron
- patients with chronic kidney disease whose kidneys no longer secrete sufficient levels of the hormone erythropoietin that promotes RBC proliferation. Erythropoietin prevents the death of cells in the erythrocyte cell line in the bone marrow. Therefore, erythropoietin allows those cells to continue to mature, exit the bone marrow and become RBCs (Jelkmann W, 2004).

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

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

Higher than Normal MCV

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

Lower than Normal MCV

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

iv) Mean corpuscular hemoglobin (MCH)

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

Higher than Normal MCH

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

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

Lower than Normal MCV

Generally, if the MCH level is below 26, this is considered too low. The MCH level can be too low because of

- blood loss over time,
- too little iron in the body,
- or Microcytic anemia which is a condition in which abnormally small red blood cells are present. Smaller red blood cells means that less hemoglobin fits in each cell.
- Hemoglobinopathy, which is a group of disorders characterized by changes in the structure of hemoglobin, can also cause a low MCH level.

v) 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.

vi)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.

1.5.4.2 White Blood Cell

White blood cells are different from red cells in the fact that they are usually larger in size 10-14 micrometers in diameter. White blood cells do not contain hemoglobin which in turn makes them translucent. Many times in diagrams or pictures white blood cells are represented in a blue color, mainly because blue is the color of the stain used to see the cells. White blood cells also have nucleii, that are some what segmented and are surrounded by electrons inside the membrane. White blood cells (leukocytes) are also known as "WBC's". White blood cells are made in the bone marrow but they also divide in the blood and lymphatic systems. They are commonly amoeboid (cells that move or feed by means of temporary projections, called pseudopods (false feet), and escape the circulatory system through the capillary beds. Normal range of WBC: 3-7×103mm3.

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.

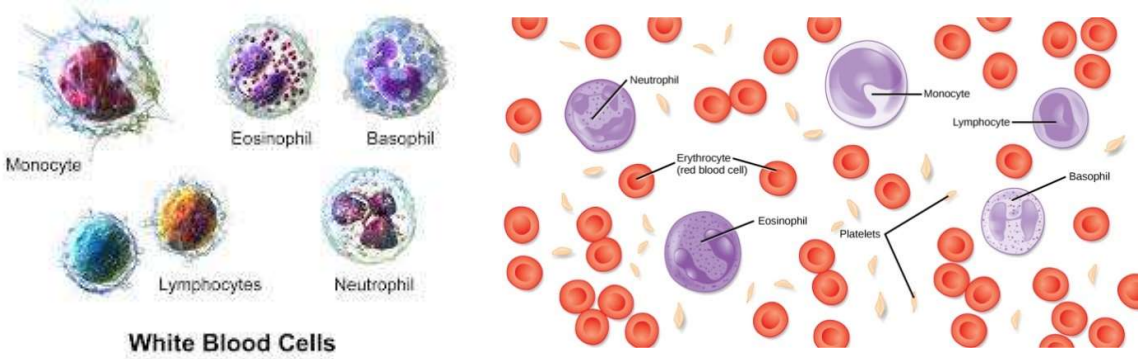


Figure-11: Different Parts of White Blood Cell and Platelet

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

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

1.5.4.3 Platelets

Platelets, also called thrombocytes, are membrane-bound cell fragments. Platelets have no nucleus, they are between one to two micrometers in diameter, and are about 1/10th to 1/20th as abundant as white blood cells. Less than 1% of whole blood consists of platelets. They result from fragmentation of large cells called Megakaryocytes - which are cells derived from stem cells in the bone marrow. Platelets are produced at a rate of 200 billion per day. Their production is regulated by the hormone called Thrombopoietin. The circulating life of a platelet is 8–10 days. The sticky surface of the platelets allow them to accumulate at the site of broken blood vessels to form a clot. This aids in the process of hemostasis ("blood stopping"). Platelets secrete factors that increase local platelet aggregation (e.g., Thromboxane A), enhance vasoconstriction (e.g., Serotonin), and promote blood coagulation (e.g., Thromboplastin).Normal range of platelet: 1000-1600×103mm3 (Ganong, 2003).

Functions:

Blood performs many important functions within the body including:

- Supply of oxygen to tissues (bound to hemoglobin, which is carried in red cells)
- Supply of nutrients such as glucose, amino acids, and fatty acids (dissolved in the blood or bound to plasma proteins(e.g., blood lipids)

- Removal of waste such as carbon dioxide, urea, and lactic acid
- Immunological functions, including circulation of white blood cells, and detection of foreign material by antibodies
- Coagulation, the response to a broken blood vessel, the conversion of blood from a liquid to a semi-solid gel to stop bleeding.
- Messenger functions, including the transport of hormones and the signaling of tissue damage
- Regulation of body pH
- Regulation of core body temperature
- Hydraulic functions

1.6 Hepatotoxicity

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

Several factors predispose the liver to xenobiotic toxicity.

- Firstly, for chemicals entering the body via the oral route, anatomical proximity to the GI-tract ensures the liver is the 'first port of call' for ingested xenobiotics.
- Secondly, chemicals and nutrients are not the only substances that enter portal blood as it perfuses the intestines: it also accumulates products of the degradation of intestinal microorganisms such as inflammogenic lipopolysaccharide components of the bacterial cell wall (i.e. endotoxin). Since endotoxin delivery may increase during xenobiotic intoxication, immunological responses to co-absorbed endotoxin can exacerbate the hepato-toxicity of ingested chemicals.
- Thirdly, in addition to entry via the portal circulation, chemicals can access the liver via arterial blood that mixes with venous blood in the hepatic sinusoids. For example, inhaled tobacco constituents that enter via the lungs are efficiently delivered to the liver via the arterial route.

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

1.6.1 Liver

The liver is a vital organ of vertebrates and some other animals. In the human it is located in the upper right quadrant of the abdomen, below the diaphragm. The liver has a wide range of functions, including detoxification of various metabolites, protein synthesis, and the production of biochemicals necessary for digestion. There is currently no way to compensate for the absence of liver function in the long term, although liver dialysis techniques can be used in the short term.

The liver is a gland and plays a major role in metabolism with numerous functions in the human body, including regulation of glycogen storage, decomposition of red blood cells, protein synthesis, hormone production, and detoxification. It is an accessory digestive gland and produces bile, an alkaline compound which aids in digestion via the emulsification of lipids. The liver's highly specialized tissue consisting of mostly hepatocytes regulates a wide variety of high-volume biochemical reactions, including the synthesis and breakdown of small and complex molecules, many of which are necessary for normal vital functions (Maton, 1993).

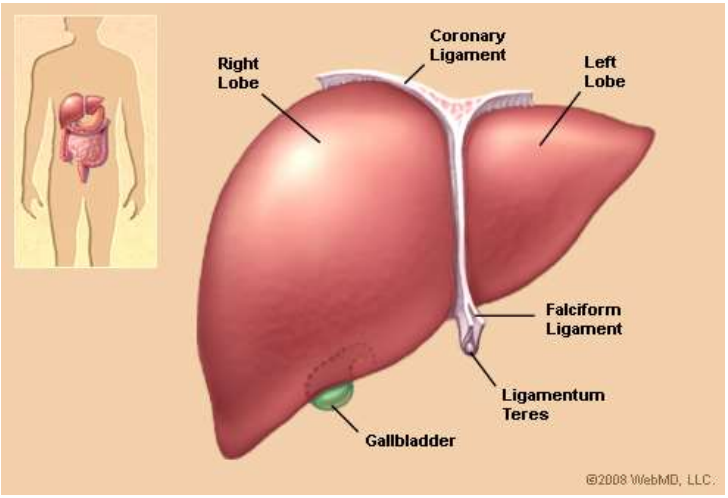


Figure-12: Anatomy of liver

Function

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

1.6.2 Liver function tests

Liver function tests (LFTs or LFs) are groups of blood tests that give information about the state of a patient's liver. These tests include prothrombin time (PT/INR), aPTT, albumin, bilirubin (direct and indirect), and others. Liver transaminases (AST or SGOT and ALT or SGPT) are useful biomarkers of liver injury in a patient with some degree of intact liver function. (McClatchey, 2002) (Mengel, 2005)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 DE 1999).

Table-6: 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

1.6.2.1 Albumin

Albumin is a protein made specifically by the liver, and can be measured cheaply and easily. It is the main constituent of total protein (the remaining from globulins). An alternative to albumin measurement is prealbumin, which is better at detecting acute changes (half-life of albumin and prealbumin is about 2 weeks and about 2 days, respectively). This test can help determine if a patient has liver disease or kidney disease, or if the body is not absorbing enough protein. Albumin helps move many small molecules through the blood, including bilirubin, calcium, progesterone, and medications. It plays an important role in keeping the fluid from the blood from leaking out into the tissues.

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

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

Increased blood albumin level may be due to:

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

1.6.2.2 Alkaline phosphatase

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

Higher-than-normal ALP levels

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

Lower-than-normal ALP levels

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

1.6.2.3 Aspartate transaminase

AST, also called serum glutamic oxaloacetic transaminase or aspartate aminotransferase, is similar to ALT in that it is another enzyme associated with liver parenchymal cells. The ratio of AST to ALT is sometimes useful in differentiating between causes of liver damage. This test is used to determine if a patient has liver damage (Nyblom H, 2004).

An increase in ALT levels may be due to:

- Cirrhosis (scarring of the liver)
- Death of liver tissue (liver necrosis)
- Hepatitis
- Lack of blood flow to the liver (liver ischemia)
- Liver tumor or cancer
- Medications that are toxic to the liver
- Pancreatitis (swollen and inflamed pancreas) (Pratt DS, 2010).

1.6.2.4 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 inflammation, such as may caused by gallstones
- a gallbladder infection
- congested blood flow through the liver due to heart failure
- liver cancer or another cancer that has spread to the liver
- taking certain medicines, such as cholesterol lowering agent, antifungal medicines, some narcotics and barbiturates, methotrexate, acetaminophen, salicylates (aspirin).

1.8. Laboratory mice

Mice are common experimental animals in biology and psychology primarily because they are mammals, and also because they share a high degree of homology with humans. They are the most commonly used mammalian model organism, more common than rats. The mouse genome has been sequenced, and virtually all mouse genes have human homolog's. They can also be manipulated in ways that are illegal with humans, although animal rights activists often object. A knockout mouse is a genetically engineered mouse that has had one or more of its genes made inoperable through a gene knockout.

There are other reasons why mice are used in laboratory research. Mice are small, inexpensive, easily maintained, and can reproduce quickly. Several generations of mice can be observed in a relatively short period of time. Mice are generally very docile if raised from birth and given sufficient human contact. However, certain strains have been known to be quite temperamental. Mice and rats have the same organs in the same places, just of different size. (MGI —2010)

2.1 Introduction of plant,*Drynaria quercifolia:*

Description:

Drynaria quercifolia is a "basket fern" belonging to the Polypodiaceae family. Commonly used name of this plant is Oak-leaf fern, the reason for its common name of "oak leaf" as the nest fronds resemble the leaves of Oaks. (Wikepidia, 2014)

In Bangladesh this plant is commonly known as Pankhiraj, Pankha, Garur. **Tribal names of this fern are** Fo-lo-rere (Marma) & Chilo vasa (Chakma). It is a epiphytic fern & it has a dimorphic digitate fronds, fertile foliage fronds and sterile nest fronds.and Rhizome which is creeping and densely clothed with red-brown scales.

(a)Rhizome and leaf (b): (b) Dimorphic digitate fronds

Figure-13 (a) Rhizome and leafof *Drynaria quercifolia.* :(b) Dimorphic digitate fronds

The dark green foliage fronds are 60-90 cm long, with elongated stalks. They are deeply lobedor pinnate,winged,and contain sori on the bottom surfaces.A sorus is a cluster of sporangia (structures containing spores) in ferns and fungi.The nest fronds are 7.5-30 cm long rounded leaves basal to the foliage fronds.They do not contain sori and are persistent, not being shed after dying. The nest fronds become green when

young, soon turning dark brown.it forms a 'basket' that collects litter and organic debris.The collected debris decomposes into humus which provides nutrients.The Rhizome of basket ferns is used to cough ,typhoid,diarrhea, migraine and headache.(Medicinal Plants of Bangladesh, 2009)

2.1 Habitat and Distribution:

Drynaria quercifolia *is a terrestrial fern and found in wet tropical environments,usually in rain forests.*They can also sometimes be found the soil among boulders ,as epiphytic on tree trunks, in man-made structures like brick walls.*Drynaria quercifolia* is native to western Australia as well as Bangladesh, India ,Southeast Asia,South China, Malaysia, Indonesia, the Philippines and New Guinea.(Ferns of Western Australia, 2013)

Hindi name of this fern is Asvakatri, Katikapan, Basingh& Malayalam name of this fern is Matilpanna, Pannakizhangu, Pannikizangu.(Philippine Medicinal Plants , 2012)

Figure-14:***Drynaria quercifolia is*** **found as epiphytic on tree trunks.**

2.1 Taxonomy:

Taxonomical hierarchy for *Drynaria quercifolia according to* NCBI (National Center for Biotechnology Information) –

Domain: Eukaryota
Kingdom: Plantae
Subkingdom: Viridaeplantae
Phylum: Tracheophyta
Subphylum: Euphyllophytina
Infraphylum: Moniliformopses
Class: Polypodiopsida .
Order: Polypodiales
Family: Polypodiaceae
Subfamily: Polypodioideae
Tribe: Drynarieae
Genus: *Drynaria*

Scientific Name:*Drynaria quercifolia* (ZipcodeZoo, 2012)

2.1 Used Parts:

Mainly Rhizomes and leaves are used as medicinal purpose.

Characteristics:

Drynaria quercifolia, commonly known as squirrel head is a non-flowering plant and belongs to the family of the Polypodiaceae.The synonym of *Drynaria quercifolia* is *polypodium quercifolium* and it is a perennial plant. The perennials reach heights of 80 to 100 centimetres. The perennial plant prefer a half-shady situation on moist soil. The more light they get, the more reddish coloration is visible in the fronds. In nature, they tend to grow attached to tree trunks. They tolerate temperatures only above at least 1°C . They absolutely require a steady supply of moisture and high humidity.*Drynaria quercifolia* is a evergreen plant. The green, imparipinnate leaves of the drynaria quercifolia are basal. The linear leaflets are petiolate.Sometimes *Drynaria quercifolia* is cultivated in house for decorative purposes.(Hortipedia, 2008)

Figure-15:A non-flowering ,evergreen plant *Drynaria quercifolia.*

Figure-16: Growing *Drynaria quercifolia* for decoration.

2.1 Life Cycle of *Drynaria quercifolia*:

Drynaria quercifolia is a spore-bearing plant. It exhibits metagenesis or the alternation of generation.[1]Metagenesis is a term used to describe the life cycle of plants in which multicellular gametophyte (haploid with *n* chromosomes) alternate with multicellular sporophyte (diploid with 2*n* chromosomes) by mitosis cell division process . Sporophytes can produce spores by meiosis process. The production of a gametophyte is not from spores, but directly from the vegetative cells of the sporophytes.The gametophytes produce male (antheridium), and female (archegonium) gametes. The gametes fuse, forming the diploid sporophyte.

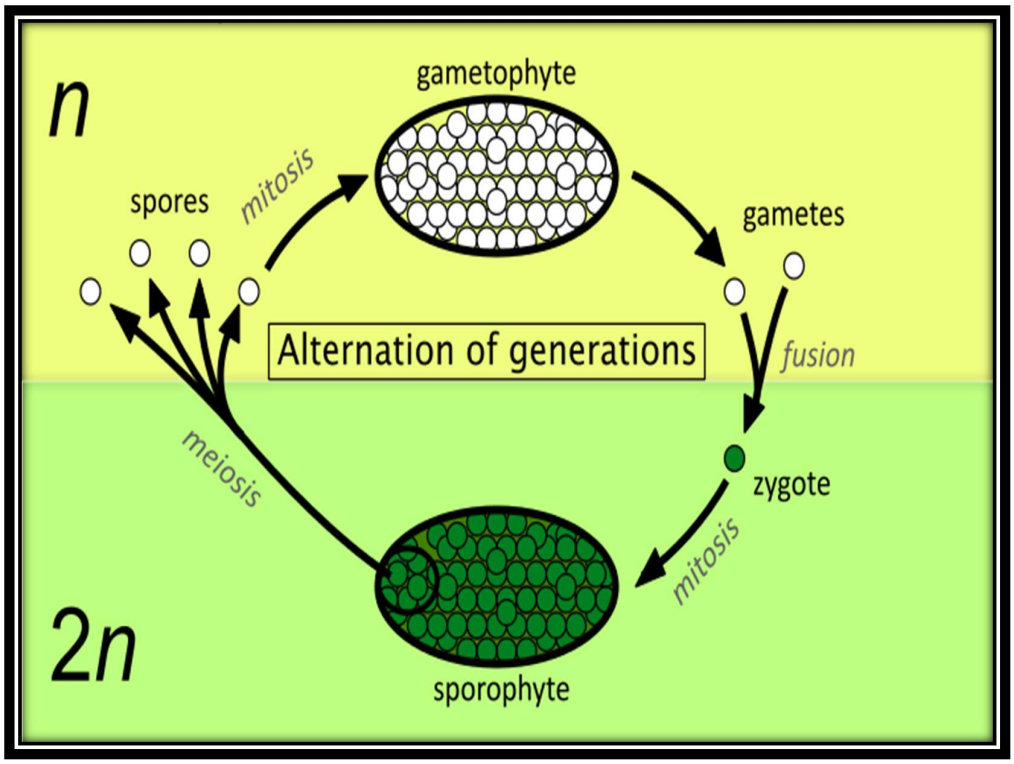


Figure-17:Diagram showing the alternation of generations between a diploid sporophyte (bottom) and a haploid gametophyte (top).

Drynaria quercifoliashows the prothallial germination in which the spores germinate into germfilament .Germ filament contains barrel-shaped chlorophullous cells with one or more rhizoids at the base cell.The tipmost cell of the germ filament can divide repeatedly by cross-walls and form a broad spatulate prothallial plate.Then One of the

cells at the top margin of the prothallial plate then divides obliquely when it has 5, 10, or more cells across its width. This results in the formation of an obconical meristematic cell. This eventually results in the formation of a notch at the anterior edge of the prothallus, giving it a roughly heart-shaped appearance. The edge of the protothallus are also usually more sparsely haired,. Their maturity comes after six to nine months and their life cycle finishes at around a year.

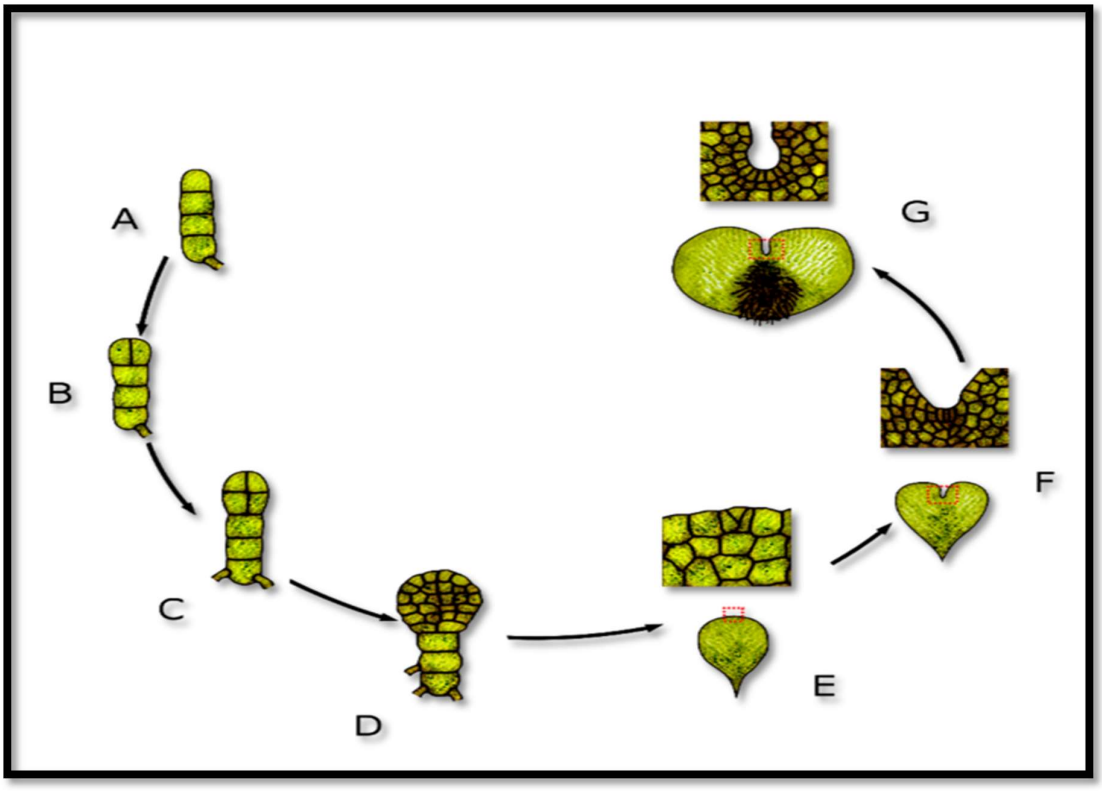


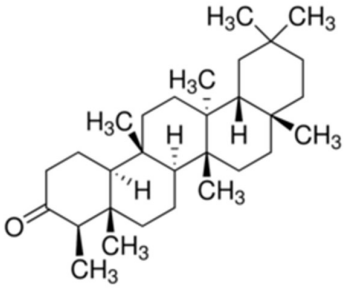
Figure-18: Prothallial germination in *Drynaria quercifolia*.

The leaves of *Drynaria quercifolia*can develop prothalli under dim light and sporophytic buds in strong light. (Wikepidia, 2014)

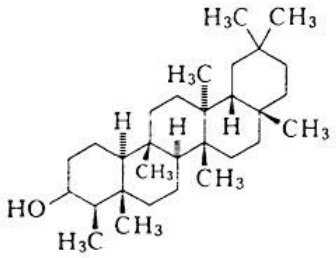
2.1 Bioactive Metabolites:

Phytochemical analysis on *Drynaria quercifoliahas* revealed a large variety of chemical compounds including phenols,tannins,alkaloids,saponins, coumarins, catechin,flavonoids, steroids, triterpenes,carboxylic acid.The plant also contains primary metabolites proteins,xanthoproteins.The Study of dried rhizomes yielded

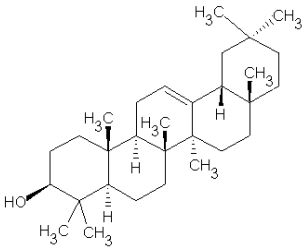
friedelin, epifriedelinol, beta-amyrin, beta-sitosterol -3-O-beta-D-glucopyranoside and naringin. .(Philippine Medicinal Plants , 2012)



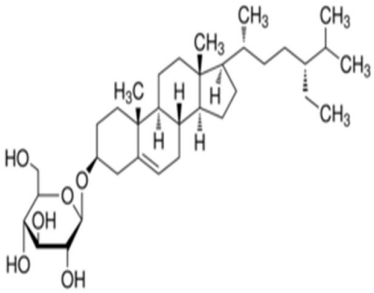
(a) Friedelin



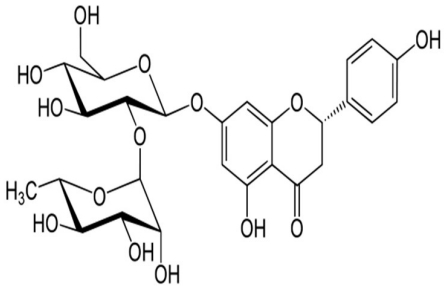
(b) Epi-friedelinol



(c) Beta-amyrin



(d) beta-sitosterol- 3-O-beta-D-glucopyranoside



(e) Naringin

Figure-19: Chemical structure ofFriedelin,Epi-friedelinol beta-sitosterol-3-O-beta-D-glucopyranoside & Naringin**.**

2.1 Medicinal Use:

Extract from the rhizomes of *Drynaria* quercifolia is used extensively in traditional medicine. Fresh or dried rhizome is used for hemoptysis In Bangladesh, rhizomes are also used in the treatment of excited mental disorders for calming effect and it also can reduce hair loss.it is also used as astringent and antihelminthic. Peeled rhizome is chewed or its juice mixed with sugar is taken in scanty urination and spermatorrhoea by the Marma tribe .The extract of the rhizome is taken by Chakma as a remedy for jaundice and dysentery. Leaves are used as poultices and used to treat fever ,dyspepsia, and phthsis,skin diseases and cough. In Tamil, Nadu the leave of *Drynaria quercifolia* used to treat arthritis. (Philippine Medicinal Plants , 2012)

Oral administration of semisolid paste of *Drynaria quercifolia*fresh rhizome and water is used during menstrual period as an antifertility agent among Kadars of Anamalai Hills Coimbatore District, Tamil Nadu. (Rajan, 1996)

2.2Literature Review:

There are few studied to conduct the activity of *Drynaria quercifolia.* Isolation and purification of compound from the plant is not conducted in large extent.The study related to the *Drynaria quercifolia* is very few.*Drynaria quercifolia* has the following biological activities like Antibacterial activity**, Anti-gonorrheal**activity**, Antipyretic**activity**, Antioxidant** activity**, Antidermatophytic, CNS Depressant Effect, Ameliorative Effect in Rheumatism, Antihelmintic,**Mosquito repellent.

Reported Bioactivity:

Antibacterial activity:

The crude extracts of DQ showed high efficiency of antibacterial activity especially to gram negative bacteria. Six different organic solvents were used to extract the bioactive compounds from the rhizome of *Drynaria quercifolia* to screen the antibacterial activity against infectious disease causing bacterial pathogens such as *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi,Salmonella paratyphi A, Salmonella paratyphi B, Salmonella marscence, Staphylococcus aureus* and *Bacillus subtilis* by agar diffusion method. The ethanolic extract of *Drynaria quercifolia* was more active against 80% of the organisms tested. It

was followed by methanolic extract (70%), benzene (50%) and chloroform extract (40%) in inhibiting the growth of the organisms tested. Petroleum ether and hexane extract of *Drynaria quercifolia* did not show any antibacterial activity against any of the pathogenic bacteria tested. (Kandhasamy*et al*, 2008)

Anti-gonorrheal activity :

The crude extract of *Ocimum sanctum*, *Drynaria quercifolia,*and *Annona squamosa* showed inhibitory activities against *Neisseria gonorrhea*, *Drynaria quercifolia* showed inhibitory activity against clinical isolates and WHO strains of Neisseria gonorrhea comparable to penicillin and ciprofloxacillin. (Shokeen*et al*, 2005)

Antipyretic activity:

The Intraperitoneal (i.p.) administration of petroleum ether and ethyl acetate soluble fractions of ethanol extract of the rhizome of *Drynaria quercifolia*, at a dose of 80 mg/kg body weight in rabbits showed antipyretic activity with significant reduction of elevated body temperature, comparable to standard Aspirin. Intraperitoneal administration of boiled milk at a was used to induce pyrexia in albino rabbit at a dose of 0.5 mL/kg body weight . (Khan*et al*, 2007)

Antioxidant activity :

Study showed the methanolic extract of powdered rhizome of Drynaria quercifolia to be an effective antioxidant compared to other extracts with significant activity compared to standard drug.((Korwar *et al*, 2010)

Antidermatophytic:

Study was done on the dermatophytic activity of four rhizome extracts from *D. quercifolia* against *T. mentagrophytes, M. canis, T. rubrum* and *E. floccosum.* Results showed the ethyl acetate extract contains triterpenes and coumarins which may be the compounds responsible for the antidermatophytic activity of this plant. (Nejad *et al*, 2014)

CNS Depressant Effect:

Study in mice of ethanol extract fractions of rhizome of *Drynaria quercifolia* showed a dose-dependent depressant effect. (Khan *et al*.,2009)

Ameliorative Effect in Rheumatism:

The present study supports the traditional claim of using D. quercifolia to treat rheumatism.. *D. quercifolia* rhizome water extract at doses of 100 and 200 mg/kg can cause reduction of paw thickness and elevation of mean body weight of arthritic rats. The treatment with crude extract showed a significant reduction in the levels of plasma and liver lysosomal enzymes as well as protein bound carbohydrates and urinary degradative collagen levels. The treatment with crude extract reduced the levels of ROS and lysosomal enzymes in neutrophils significantly. The significant reduction in the levels of serum pro-inflammatory cytokines (TNF-α and IL-1β) and the increment in the levels of anti-inflammatory cytokine (IL-10) were also observed with the treatment. (Saravanan.*et al*, 2013)

Antibacterial Efficacy Against Urinary Tract Pathogens:

Various extracts were tested for antibacterial activity against clinically isolated urinary tract pathogens. An acetone extract of *Drynaria quercifolia* was effective against *Enterococcus faecalis* and *Streptococcus pyogenes* while an ethanol extract was effective against *Pseudomonas aeruginosa*.((Mithraja.*et al.*,2012)

Antihelmintic:

Study evaluated the anthelmintic potential of *D. quercifolia* against adult earthworms and piperazine citrate as standard. Results showed anthelmintic potency of the extract, inversely proportional to the time taken for paralysis and death of the worms. (Kulkarni *et al,* 2010)

Antibacterial / 3,4- dihydroxybenzoic acid:

Study shows that 3,4- dihydroxybenzoic acid was isolated from the rhizome of D. quercifolia which showed significant antibacterial activity against four gram-positive and six gram-negative bacteria.

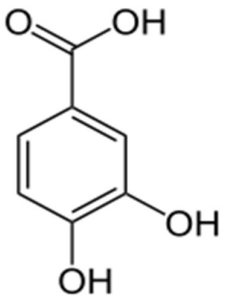


Figure-20:3,4-dihydroxybenzoic acid. (Khan *et al*,2007)

Mosquito repellent:

D. quercifolia extracts also exhibit high repellency (as high as 90 to 100%) to adult female Mosquito species *C. quinquefaciatus* and *Aedis aegypti* with increase in concentration of the extract. .Petrolyum ether extract is very effective and it showed the 'Knock down' effect within 20 min at 160 mg. (Marathe *et al*,2011)

Figure 21:*Drynaria quercifolia*

3 MATERIALS & METHODS:

3.1. Plant Materials:

3.1.2. Collection:

Drynaria quercifoliarhizomes are collected from Barishal District of Bangladesh and identified by the National Herbarium of Bangladesh,where a voucher specimen is conserved under the reference number DACB Accession number 38478.

Figure-22: Identification of *Drynaria quercifolia* by the National Herbarium of Bangladesh.

3.1.3. Solid liquid Extraction:

Extraction is first pre-purification step in the isolation of active compounds of a medicinal plant. At first powdered plant material(2.5kg or2500g) is mixed with extractant (12.5L of methanol) with continuous stirring or shaking at room temperature (72 hrs×3times).

Solvent from sample is filtered with white cotton cloth and filter papers and evaporated off under reduced pressure and below 50°C temperature in a rotary evaporator to obtain 84.79gm of crude extract.a yield of 8.479% of crude extract is obtained. Then the plant extract is stored in refrigerator at 4°C to avoid negative effect of light and temperature.

Figure-23: 2nd time filtration done with filter paper

Figure-24: Evaporation of methanol by Rotary evaporator

Figure-25: Collection of crude extract.

3.1.4Liquid-liquid Extraction

The crude extract was subjected to liquid-liquid extraction using three solvent systems, N-hexane, Dichloromethane (DCM) and water system. At first 22 gm of crude extract was mixed with distilled water and methanol. Then added N-hexane to the mixer and mixed properly. Then it poured into a separating funnel. After some time two layers was separated.

- ✓ bottom layer was N-hexane layer
- ✓ Upper layer was Water layer

Then we collected the bottom layer slowly and subjected to evaporation to get N-hexane extract of *Drynaria quercifolia*

In the upper water layer some distilled water and Dichloromethane (DCM) was added and mixed properly.Then it poured into a separating funnel. After some time two layers was separated.

- ✓ Bottom layer was Water layer
- ✓ Upper layer was Dichloromethane layer

Then we collected the Upper layer slowly and subjected to evaporation to get Dichloromethane extract of. *Drynaria quercifolia*

Figure-26:Liquid-liquid extraction (*drynaria quercifolia*)

Figure-27: Collection of fraction

3.1.5. Experimental Animals:

Swiss albino mice of either sex (22-28gm) are obtained from the Animal Resource Division of International Center for Diarrhoeal Disease and Research,Bangladesh (ICDDRB). The mice are kept in animal house at a standard environmental condition (temperature 22 ± 1°C,relative humidity 55 ± 5% and 12h light/ 12 hr dark cycle).Animals are provided standard food (ICDDRB) and clean water ad libitum. Animals are treated in accordance with the current law and the NIH Guide for Care and Use of Laboratory Animals.

Figure-28:***Swiss albino*** **mice kept in the cage with adequate food and clean drinking water.**

3.1.6. Analgesic Activity Test:

3.1.7. Materials for Analgesic Activity Test:

- Beaker 1 liter
- Analytical Balance,
- Feeding needle: 1 c.c.
- Insulin syringes 100 units both disposable and nondisposable.

3.1.8. Chemical Agents Used in Analgesic activity Test:

- 5% CMC (Vehicle) 10ml/kg as negative control,
- 0.7% v/v, Acetic acid in 0.9% saline (10ml/kg).

3.1.9..Standard Drugs Used in Analgesic activity Test:

- Indomethacin (10mg/kg) used as positive control in acetic acid induced writhing test.

3.1.10. Doses Used in Different Experiments (Analgesic activity Test):

Analgesic Activity of the Extract:

i) Acetic Acid induced Writhing Test:

Ethanolic extracts of*Drynaria quercifolia*at a dose of 100mg/kg, 200mg/kg and400 mg/kg of the ofare administered orally. 5% CMC was used as a vehicle with plant ethanolic extract for preparing different doses.

3.1.11. Methods for Analgesic Activity Test:

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

- Acetic Acid Induced Writhing 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.

Acetic Acid Induced writhing method:

Acetic acid induced writhing method is adopted for evaluation of analgesic activity. Writhing is defined as a stretch, tension to one side, extension of hind legs, contraction of the abdomen so that the abdomen of mice touches the floor, turning of trunk (twist). Any writhing is considered as a positive response.

The analgesic activity of samples are evaluated using acetic acid induced writhing test. At first *Swiss albino* mice of either sex are divided into 4 groups , named group-1, group-2, group-3, group-4,. Each group contains 6 mice (n=6). After an over night fast, 5% CMC is given orally (at dose 10ml/kg) to group-1 (considered as negative control). Then n-haxen fraction plant extract (suspended in 5% CMC vehicle) is given orally to group-2, and dichloromethen fraction group-3 at dose 200mg/kg (as per body wt of mice) respectively. After 30 min of oral drug administration, 0.7% v/v acetic acid solution(Dose :0.1ml/10gm as per body wt of mice) is injected intraperitonially to the group-1,group-2 & group-3

Figure-29: Process of Intra-peritoneal injection to mice.

Indomethacin (Dose:10 mg/kg) is given orally to group-4(considered as positive control). After 15 min of oral drug administration,0.7% v/v acetic acid solution is injected intraperitonially to the group-4. After 5 min of acetic acid administration, number of writhing is counted for 10min. Full writhing is not always accomplished by the animal.This incomplete writhing is considered as half-writhing.Two half writhing are taken as one full writhing. Percentage inhibition of writhing is also calculated .Percent reduction indicates the percentage protection against abdominal constriction which is taken as an index of analgesia.

It is calculated as:

$\{(W_c - W_t) \times 100\}/W_c$

Where, W_c = number of writhing of the control group

W_t = number of writhing of the treated group. (Zulfiker*et al*., 2010)

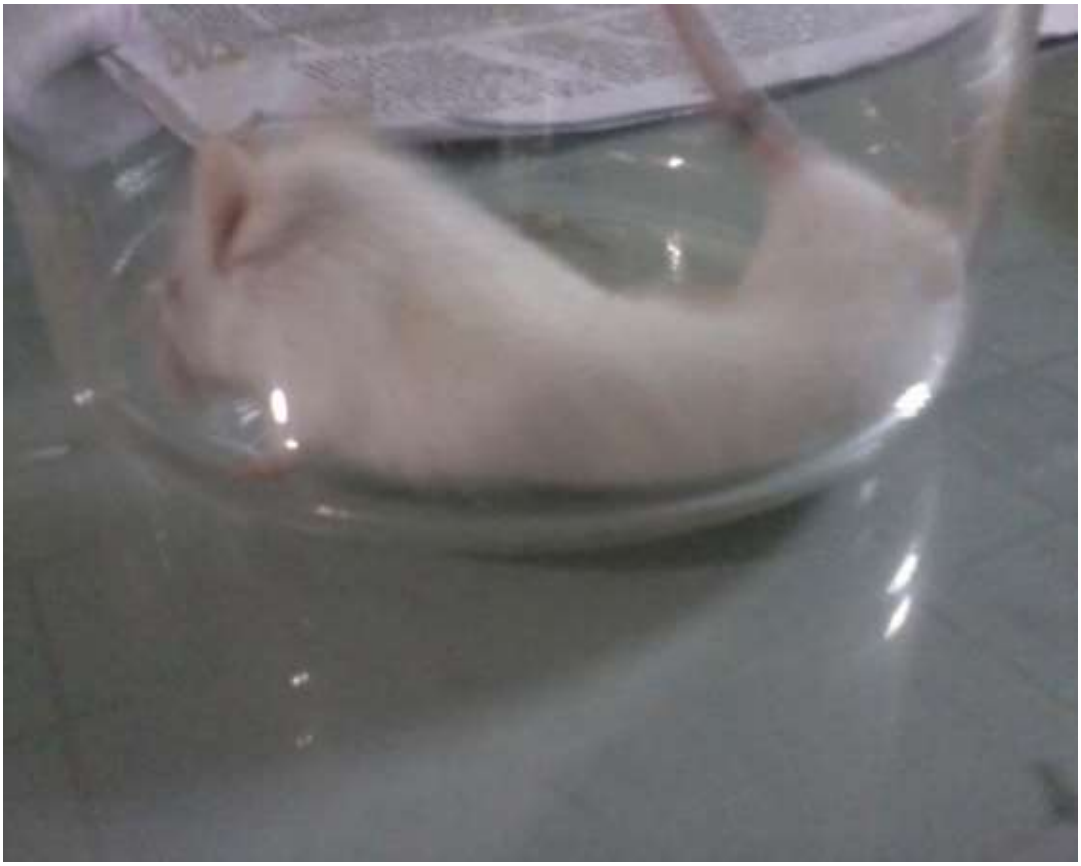


Figure-30:Abdominal Constriction of Mice after Intraperitoneal Injection of Acetic Acid.

3.2.1. CNS Activity Test:

3.2.2. Materials for CNS Activity Test:

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

3.2.3. Chemical Agents Used in Analgesic activity Test:

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

3.2.4. Standard Drugs Used in CNS activity Test:

- Diazepam 1mg/kg used as positive control in open field test.
- Diazepam 1mg/kg used as positive control in hole board test.

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

i) Open Field Test:

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

ii) Hole Board Test:

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

3.2.6. Methods for CNS Activity Test:

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

- Open Field Test.
- Hole Board Test.

After the extraction of the plant, each group is treated with the extract in order to determine some specific parameters according to the experimental protocol.

Open Field Test:

In this experiment, the method according to Gupta, 1971 was employed. An open field, a test paradigm which is highly standardized to evaluate locomotor activity (Kelley, 1993).The animals was divided into negative control, positive control and test groups containing six mice in each group. Negative control group received vehicle (5% CMC solution) at a dose of 10 mg/kg body weight orally. The test groups received extracts of *Drynaria quercifolia* at the doses of 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-31: Open-field test apparatus

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 *Drynaria quercifolia* at the doses of 400 an8d 00mg/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 30 minutes intervals and were compared with the control animals.

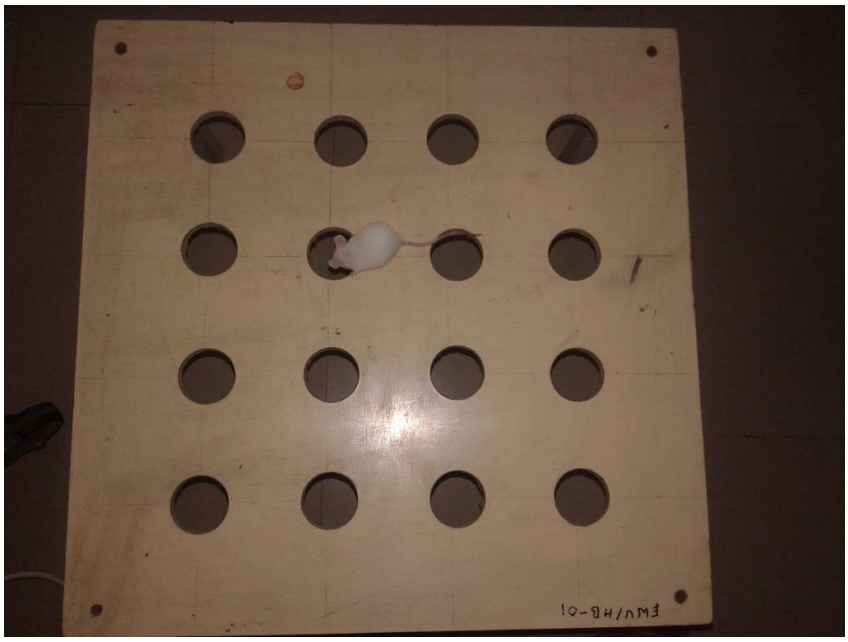


Figure:31Hole Board Test apparatus

3.3.1. Toxicity Test

3.3.2. 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
- Eppendorf tubes
- 250 ml food grade plastic pot
- Gloves
- Mask

3.3.3. Chemical Agents Used Toxicity Test

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

3.3.4. Doses Used for Toxicological Activity of the Extract:

i) Acute Toxicity Test:

Methanolic extracts of *Drynaria quercifolia* at a dose of 1000mg/kg, 2000mg/kg 3000mg/kg 4000mg/kg and 5000mg/kgwere administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses.

ii) Sub-chronic Toxicity Test:

Methanolic extracts of*Drynaria quercifolia* at a dose of 200mg/kg, 400mg/kg and600 mg/kg of theofare administered orally. 5% CMC is used as a vehicle with plant methanolic extract for preparing different doses.

3.3.5. Methods for Acute and Sub-chronic Toxicity Test:

Acute Toxicity Test

The acute toxicity of in Swiss albino mice was studied as reported method. Each extract were given to four groups (n = 6) of mice at 2000 and 3000 mg/kg body weight, orally. The treated animals were kept under observation for 3 days, for mortality and general behaviour. (Nirankush Paul, 2012)

Sub-chronic Toxicity Test

The adult Swiss albino mice were divided into four groups containing 6 animals per group. The first group received 5% CMC (Vehicle) 10ml/kg and the other three groups received the three doses of extracts like 200 mg/kg, 400 mg/kg, 600 mg/kg according to body weight orally, respectively daily for 45 consecutive days. Food and water intake of animals were observed during this period. Body weight was taken for every 3 days. Twenty four hours after the last dose (i.e., at the 44th day), the mice were fainted by using chloroform and collected blood using 5 ml disposable syringe from cardiac puncture and reserved it in both heparinized and non- heparinized Epindrop tube. Then also collected other organ like Brain, Liver, Kidneys, Heart, Lung, and Stomach and reserved it food grade plastic pot having 5% formalin. Then this blood and liver was used for the study of Hematology test, Protein Test and Liver biochemical parameters Test. (Nirankush Paul, 2012)

Hematological parameters

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

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.

Histopathological studies

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

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.

3.4. Larvicidal test method :

Three concentration (1mg, 3mg, 5mg) dose was given.20 mosquito larvae(*Culax pipin*) was placed in each cup. Control test was also conducted. There were 3 replications for each concentration of plant extracts. Numbers of dead larvae on each cup were counted for 5 days.(WHO

Figure 32: larvae (*Culax pipin*in microscopic camera view*)*

Figure 33: larvae (*Culax pipin)*

Figure 33: Breeding chamber

Figure 35:experiment in progress

4.1 Analgesic Activity Test of *Drynaria quercifolia:*

Acetic Acid Induced Writhing Test on Mice:

Analgesic activity of the methanolic crude extracts and fractions of *Drynaria quercifolia* carried out in these studies. A total 2 fractions (N-hexane fraction and Dichloromethane fraction) were subjected for the evaluation of the analgesic activities**.** The experimental findings that are noted as follows:

4.1.1 Analgesic Activity Test of Methanolic Crude extract of *Drynaria quercifolia:*

Analgesic activity of the methanolic extracts of *Drynaria quercifolia* carried out in these studies. The experimental findings that are noted as follows:

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

This group of animals only received vehicles (5% CMC) 10 ml/kg orally. The observed total writhing count is followed with a mean value of 63.84±0.95 (Mean ±SEM) during 10 minutes observation.

Test Group-1 (Plant Extract, 100mg/kg)

In this case of the test group of the mice receive the *Drynaria quercifolia* methanolic extract with a dose of 100 mg/kg orally. The total number of writhing count is followed with a mean value of 57.00±0.58 (Mean ±SEM) during 10 minutes of observation. The 100 mg/kg dose of *Drynaria quercifolia* extract shows a significant decrease in the total number of writhing count. The standard drug Indomethacin showed 62.92 % inhibition in the total number of writhing count. The percent inhibition of writhing by this group which was pretreated with *Drynaria quercifolia*(at a dose 100mg/kg as per body weight of mice) is 10.71% which is 52.21% lower than the Indomethacin.

Test Group-2 (Plant Extract, 200mg/kg)

In this case of the test group of the mice receive the *Drynaria quercifolia* methanolic extract with a dose of 200 mg/kg orally. The total number of writhing count is followed with a mean value of 47.00±0.58 (Mean ±SEM) during 10 minutes of observation. The 200 mg/kg dose of *Drynaria quercifolia* extract shows a significant decrease in the total number of writhing count. The standard drug Indomethacin showed 62.92 % inhibition in the total number of writhing count. The percent inhibition of writhing by

this group which was pretreated with *Drynaria quercifolia*(at a dose 200mg/kg as per body weight of mice) is 23.37% which is 39.55% lower than the Indomethacin.

Test Group-3 (Plant Extract, 400mg/kg)

In this case of the test group of the mice receive the *Drynaria quercifolia* methanolic extract with a dose of 400 mg/kg orally. The total number of writhing count is followed with a mean value of 34.00±0.77 (Mean ±SEM) during 10 minutes of observation. The 400 mg/kg dose of *Drynaria quercifolia* extract shows a significant decrease in the total number of writhing count. The standard drug Indomethacin showed 62.92 % inhibition in the total number of writhing count. The percent inhibition of writhing by this group which was pretreated with *Drynaria quercifolia*(at a dose 400mg/kg as per body weight of mice) is 45.95% which is 16.97% lower than the Indomethacin.

Positive Control Group (Indomethacin, 10mg/kg)

This group of mice receive the standard drug Indomethacin of 10mg/kg orally. The observed total number of writhing count is followed with a mean value of 23.67±0.89 (Mean ±SEM) during 10 minutes observation.The observed P value is 0.000. The positive control shows a highly significantly decrease in the total number of writhing count.The percent inhibition of writhing by this group that is pretreated with *Drynaria quercifolia* (at a dose 10mg/kg as per body weight of mice) is 62.92% when compared to the negative control .

Table–5: Analgesic Activity of plant extract by Acetic Acid Induced Writhing test in Mice.

Groups	Treatment	Dose	No.of writhing	Percent inhibition
Negative control	5% CMC	10ml/kg	63.84±0.95	-
Group-1	Crude extract of *Drynaria quercifolia*	100mg/kg	57.00±0.58***	10.71%
Group-2	Crude extract of *Drynaria quercifolia*	200mg/kg	47.00±0.58***	26.37%
Group-3	Crude extract of *Drynaria quercifolia*	400mg/kg	34.50±0.77***	45.95%
Positive control	Indomethacin	10mg/kg	23.67±0.89***	62.92%

Each value is the mean ± SEM for 6 mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

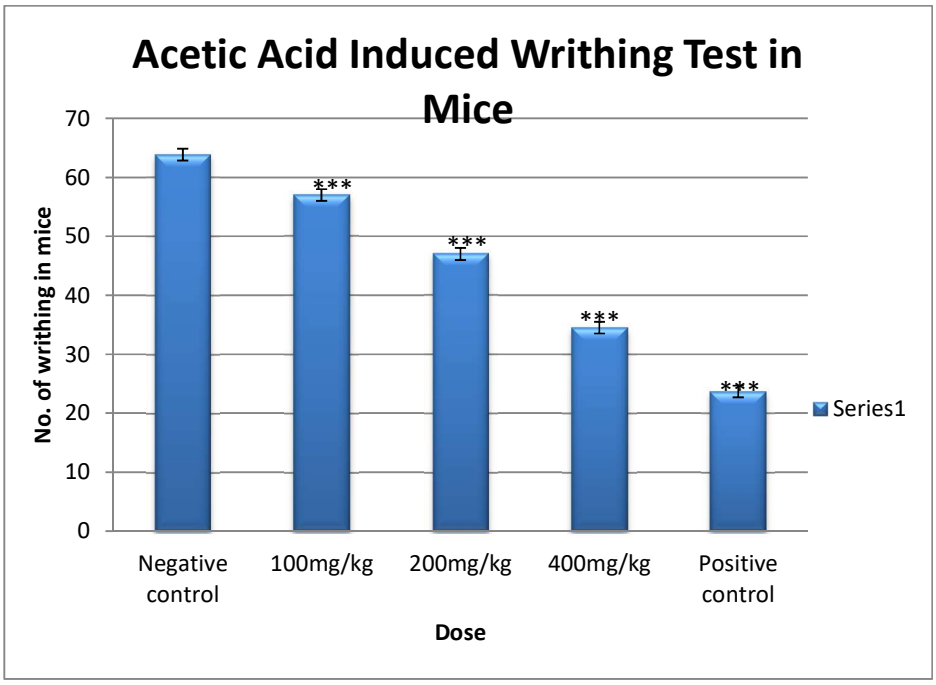


Figure-: Bar diagram showing the dose dependent effect of *Drynaria quercifolia* rhizome on acetic acid induced writhing response in mice .(Values are the mean±SEM,n=6.) .

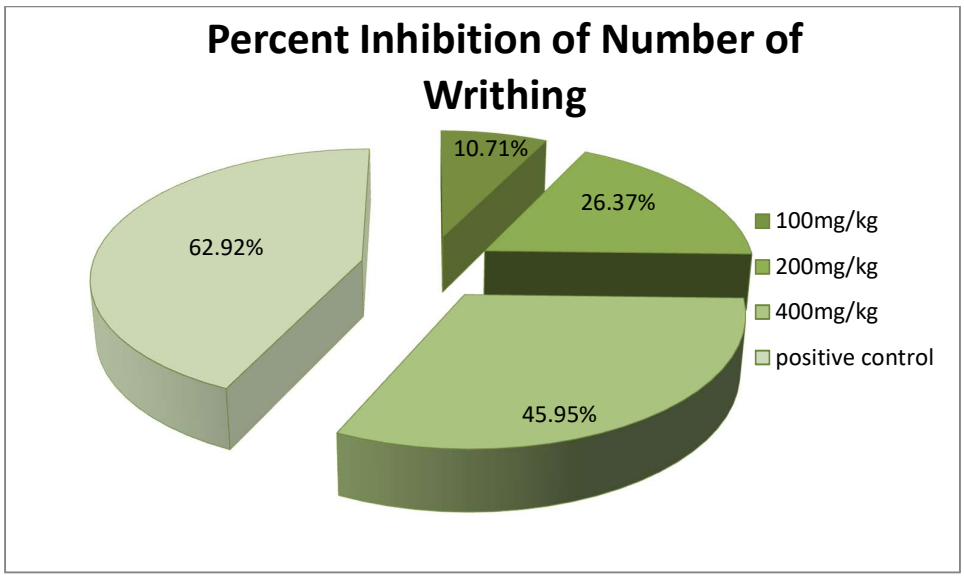


Figure-: Pie diagram showing the dose dependent effect of ethanolic extract of *Drynaria quercifolia* (values are the inhibition percentage of total number of writhing compared with negative control).

4.1.2 Analgesic Activity Test of fractions of *Drynaria quercifolia:*

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

This group of animals only received vehicle (5% CMC) 10 ml/kg orally. The observed total writhing count is followed with a mean value of 54.33±0.88 (Mean ±SEM) during 10 minutes observation.

Total Number of writhing count and Percent Inhibition of Writhing:

Test Group-1 (N-hexane Fraction of *Drynaria quercifolia:* 200mg/kg)

In this case of the test group of the mice receive the N-hexane fraction of the plant extract with a dose of 200 mg/kg orally. The total number of writhing count is followed with a mean value of 20.50±1.11 (Mean ±SEM) during 10 minutes of observation. The 200 mg/kg dose of N-hexane fraction of*Drynaria quercifolia*extract shows a significantdecrease in the total number of writhing count. The standard drug Indomethacin showed72.47% inhibition in the total number of writhing count. The percent inhibition of writhing by this group which was pretreated with *Drynaria quercifolia*(at a dose 400mg/kg as per body weight of mice) is 80.67%which is 8.2% greater than the Indomethacin.

Test Group-2 (Dichloromethane Fraction of *Drynaria quercifolia* Extract: 200mg/kg)

In this case of the test group of the mice receive the Dichloromethane fraction plant extract of 200 mg/kg orally. The total number of writhing count is followed with a mean value of 2.8±.68 (Mean ±SEM) during 10 minutes observation. The 200 mg/kg dose of Dichloromethane fraction of *Drynaria quercifolia*extract shows a significant decrease in the total number of writhing count. The standard drug Indomethacin showed 72.47% inhibition in the total number of writhing count. The percent inhibition of writhing by this group which was pretreated with *Drynaria quercifolia* (at a dose 400mg/kg as per body weight of mice) is 96.27% which is 23.8% greater than the Indomethacin.

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Positive Control Group (Indomethacin, 10mg/kg)

This group of mice receives the standard drug Indomethacin of 10mg/kg orally. The observed total number of writhing count is followed with a mean value of 18.67±1.02(Mean ±SEM) during 10 minutes observation. The positive control shows a highly significantly decrease in the total number of writhing count. The percent inhibition of writhing by this group that is pretreated with ***Drynaria quercifolia***(at a dose 10mg/kg as per body weight of mice) is 65.63%when compared to the negative control.

Table–7: Analgesic Activity of fractions of *Drynaria quercifolia* by Acetic Acid Induced Writhing test in Mice.

Groups	Treatment	Dose	No. of writhing	Percent inhibition
Negative control	5% CMC	10ml/kg	67.83±1.64***	-
Group-1	N-hexane fraction of plant extract	200mg/kg	20.50±1.11***	69.77%
Group-2	DCM fraction of plant extract,	200mg/kg	2.8± 0.68***	96.27%
Positive control	Indomethacin	10mg/kg	18.67±1.02***	72.47%

Each value is the mean ± SEM for 6 mice, * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

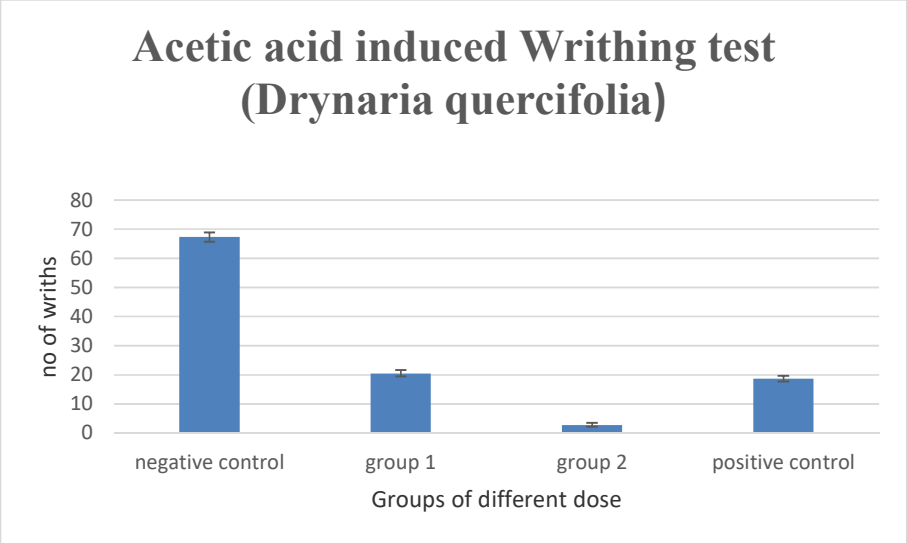


Figure-36:Graphical Presentation of Analgesic Activity of plant extract of *drynaria quercifolia* by Acetic Acid Induced Writhing test in Mice.

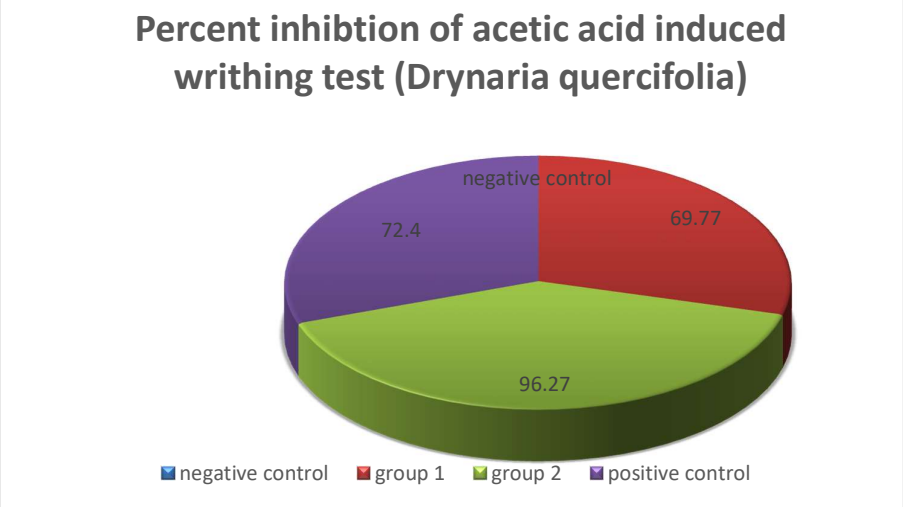


Figure-37:Percent inhibitionof Analgesic Activity of plant extract of *drynaria queiarcifolia* by Acetic Acid Induced Writhing test in Mice.

4.2.1CNS Activity Test of Methanolic Extract of *Drynaria quercifolia* Open Field Test:

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

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

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

- This group of animals only received vehicle (5% CMC) 10 ml/kg orally.
- The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 121.50±1.05***, at 30 min 121±2.62*** at 60 min 118±0.56***, at 90 min119.83±0.79*** and at 120 min 121.83±1.10*** (Mean ±SEM) during 2 minutes observation.

- The observed total number of central locomotion count is followed with a mean value of at 0 min 22.67±1.22***, at 30 min 20.67±0.91*** at 60 min 21.50±0.92***, at 90 min 21.0±0.57***and at 120 min 121.83±1.10*** (Mean ±SEM) during 2 minutes observation.

- The observed total number of leaning count is followed with a mean value of at 0 min 20.33±0.76***, at 30 min 21.16±2.13***at 60 min 21.33±0.80***, at 90 min 17.67±0.76*** and at 120 min 21.33±0.80*** (Mean ±SEM) during 2 minutes observation.

- The observed total number of rearing count is followed with a mean value of at 0 min 0.67±0.33*, at 30 min 0.67±0.33*at 60 min 0.16±0.16*, at 90 min 0.67±0.21** and at 120 min 1.00±0, 25** (Mean ±SEM) during 2 minutes observation.

- The observed total number of grooming count is followed with a mean value of at 0 min 0.50±0.22** at 30 min 0.50±0.22** at 60 min 0.50±0.34** at 90 min 0.67±0.21** and at 120 min 0.66±0.21** (Mean ±SEM) during 2 minutes observation.

- The observed total number of defecation count is followed with a mean value of at 0 min 0.67±0.21** at 30 min 0.67±0.21** at 60 min 0.50±0.22** at 90 min

0.83±0.30** and at 120 min 0.83±.16*** (Mean ±SEM) during 2 minutes observation.

4.2.1Test Group-1 (Plant Extract, 400mg/kg)

- This test group of mice receive the plant extract of 400 mg/kg orally.
- The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 107±2.7***, at 30 min 50.16±1.7***, at 60 min 29.8±1.8***, at 90 min 17.8±1.59*** and at 120 min 13.4±1.3*** (Mean ±SEM) during 2 minutes observation.

- The observed total number of central locomotion count is followed with a mean value of at 0 min 16.00±0.58***, at 30 min 3.00±0.54*** at 60 min 3.2±0.96***, at 90 min 3.67±1.3*** and at 120 min 3.20±0.48*** (Mean ±SEM) during 2 minutes observation.

- The observed total number of leaning count is followed with a mean value of at 0 min 18.67±0.49***, at 30 min 15.16±0.70***, at 60 min 12.0±0.57***, at 90 min 9.50±0.56*** and at 120 min 7.5±0.76*** (Mean ±SEM) during 2 minutes observation.

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

- The observed total number of grooming count is followed with a mean value of at 0 min 0.6±0.4** at 30 min 27.60±3.6*** at 60 min 0.34±1.4** at 90 min 15.4±1.02** and at 120 min 5±0.90(Mean ±SEM) during 2 minutes observation.

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

4.2.1Test Group-2 (Plant Extract, 800mg/kg)

- These groups of mice receive the plant extract of 800 mg/kg orally.
- The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 106.83±5.32***, at 30 min 12.00±1.00***, at 60 min 8.00±0.31***, at 90 min 5.8±0.91*** and at 120 min 4.8±.37*** (Mean ±SEM) during 2 minutes observation.

- The observed total number of central locomotion count is followed with a mean value of at 0 min 18.00±.73***, at 30 min 2.8±0.58***, at 60 min 2.8±0.40***, at 90 min 2.6±0.40*** and at 120 min 2.2±0.60*** (Mean ±SEM) during 2 minutes observation.

- The observed total number of leaning count is followed with a mean value of at 0 min 13.83±1.2***, at 30 min 2.6±0.74***, at 60 min 0.2±0.2***, at 90 min .2±0.2***, and at 120 min 0(Mean ±SEM) during 2 minutes observation.

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

- The observed total number of grooming count is followed with a mean value of at 0 min 0.6±0.40** at 30 min 32.4±1.4*** at 60 min 27.4±2.01*** at 90 min 9.00 ±0.70** and at 120 min1.6±0.50*** (Mean ±SEM) during 2 minutes observation.

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

4.2.1Positive Control Group (Diazepam, 1mg/kg)

- This group of mice receives the standard drug Indomethacin of 10mg/kg orally.
- The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 111.67±1.3***, at 30 min 69.0±1.06***, at 60 min 50.0±1.54***, at 90 min 26.83±1.19*** and at 120 min 15.0±1.41*** (Mean ±SEM) during 2 minutes observation.

- The observed total number of central locomotion count is followed with a mean value of at 0 min 19.16±1.01***, at 30 min 9.83±0.79***, at 60 min 4.16±0.47***, at 90 min 3.00±0.51*** and at 120 min 2.0±0.25*** (Mean ±SEM) during 2 minutes observation.

- The observed total number of leaning count is followed with a mean value of at 0 min 18.33±0.42***, at 30 min 9.0±0.57***, at 60 min 7.5±0.42***, at 90 min 5.0±0.36*** and at 120 min 2.83±0.54*** (Mean ±SEM) during 2 minutes observation.

- The observed total number of rearing count is followed with a mean value of at 0 min1.0±0.36**, at 30 min 0, at 60 min 0, at 90 min 0 and at 120 min 0 (Mean ±SEM) during 2 minutes observation.

- The observed total number of grooming count is followed with a mean value of at 0 min 1.0±0.25** at 30 min 0, at 60 min 0, at 90 min 0 and at 120 min 0 (Mean ±SEM) during 2 minutes observation.

- The observed total number of defecation count is followed with a mean value of at 0 min 0.83±0.30** at 30 min 1.0±0.36**, at 60 min 1.16±0.30**, at 90 min 0.33±0.21** and at 120 min 0.50±0.22** (Mean ±SEM) during 2 minutes observation.

Table8: CNS Activity of plant extract of *Drynaria quercifolia* 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	121.50±1.05***	121±2.62***	118±0.56***	119.83±0.79***	121.83±1.10***
Crude extract of *Drynaria quercifolia*	400mg/kg	107±02.7***	50.16±1.7***	29.8±1.8***	17.8±1.59***	13.40±1.3***
Crude extract of *Drynaria quercifolia*	800mg/kg	106±5.32***	12.50±1.00***	8.00±0.31***	5.8±0.91***	4.8.±0.37***
Positive control, Diazepam	1mg/kg	111.67±1.33***	69.0±1.06***	50.0±1.54***	26.83±1.19***	15.0±1.41***

Each value is the mean ± SEM for 5mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

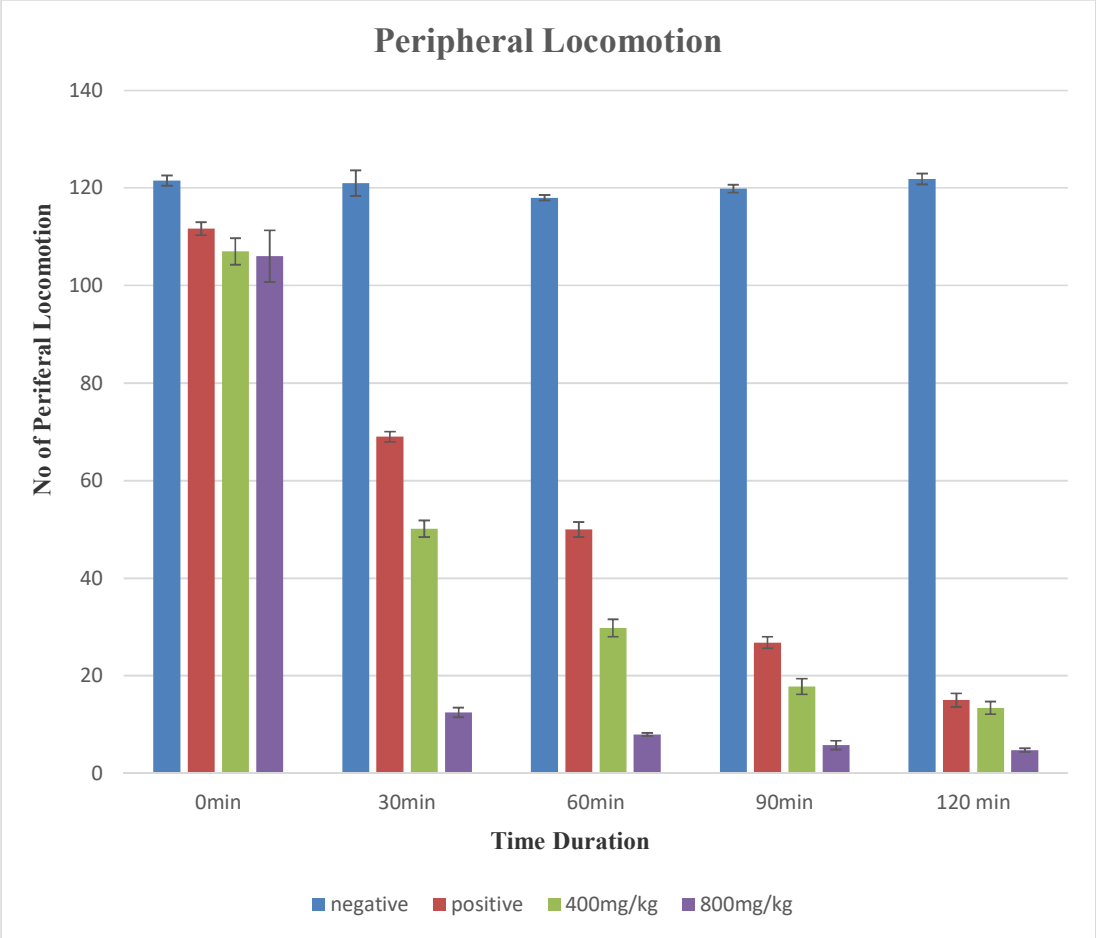


Figure38: Graphical Presentation of CNS Activity of plant extract of *Drynaria quercifolia* by Open Field Test (Peripheral Locomotion) in Mice.

Table9: CNS Activity of plant extract of *Drynaria quercifolia* 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	22.67±1.22***	20.67±0.91***	21.50±0.92***	21.0±0.57***	23.0±1.31***
Crude extract of *Drynaria quercifolia*	400mg/kg	16.00±0.58***	3.0±0.54***	3.2±0.96***	3.6±1.3***	3.2±0.48***
Crude extract of *Drynaria quercifolia*	800mg/kg	18±.73***	2.8±0.58***	2.8±0.40***	2.6±0.40***	2.2±0.2***
Positive control, Diazepam	1mg/kg	19.16±1.01***	9.83±0.79***	4.16±0.47***	3.00±0.51***	2.0±0.25***

Each value is the mean ± SEM for 5mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

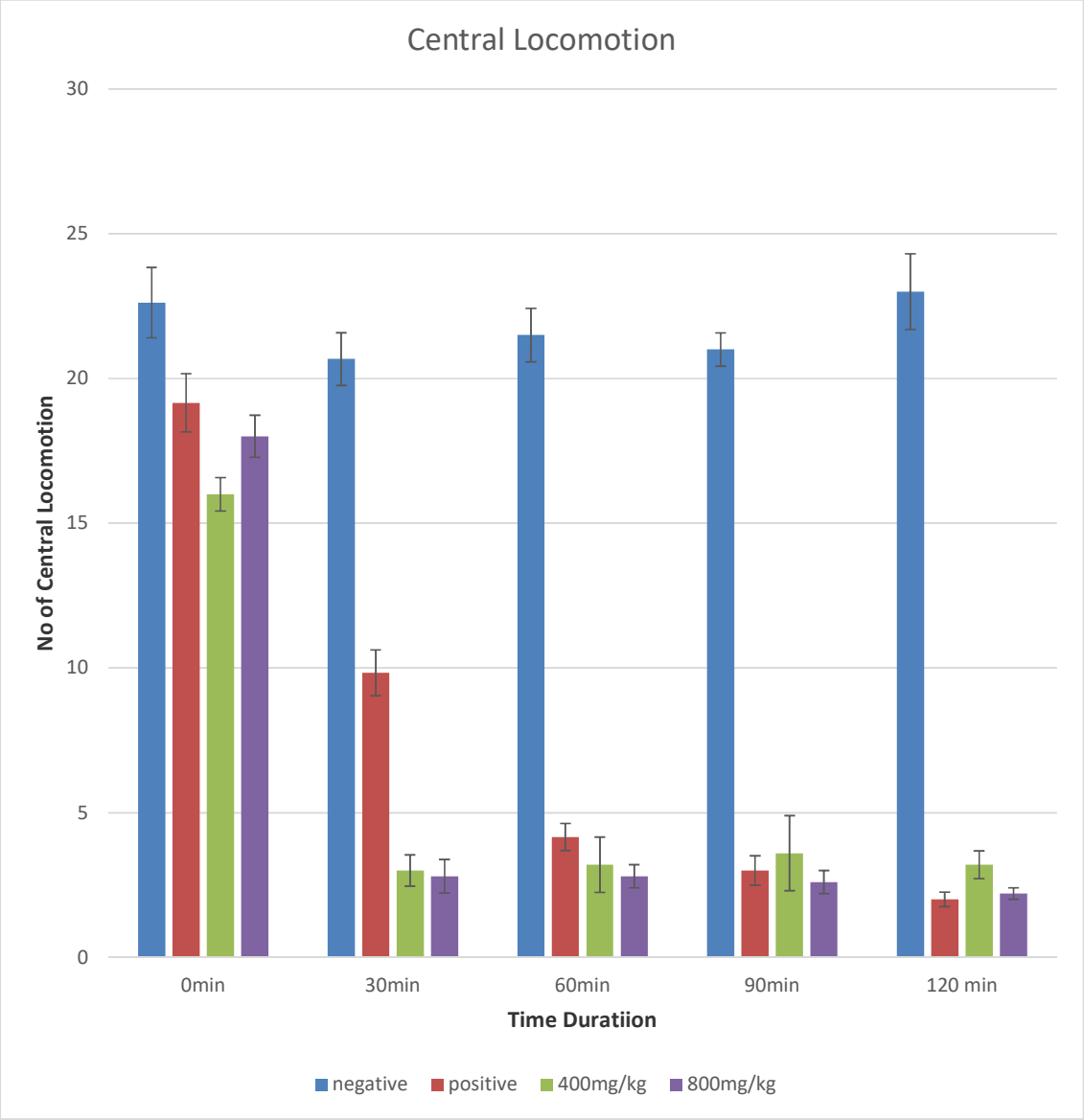


Figure-39: Graphical Presentation of CNS Activity of plant extract of *Drynaria quercifolia* by Open Field Test (Central Locomotion) in Mice.

Table10: CNS Activity of plant extract of *Drynaria quercifolia* by Open Field Test (grooming) in Mice.

Groups	Dose	No. of grooming				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	.50±0.22***	.50±0.22***	0.50±0.22***	.67±0.22***	0.46±0.21***
Crude extract of *Drynaria quercifolia*	400mg/kg	.67±0.49***	27.60±3.60***	34.0±1.87***	1.50±1.02***	0.5±0.90***
Crude extract of *Drynaria quercifolia*	800mg/kg	.83±0.94***	32.4±1.4***	27.4±2.01***	9.00±0.70***	1.6±0.50***
Positive control, Diazepam	1mg/kg	1.0±0.25**	1.0±0.25**	1.0±0.25**	1.0±0.25**	1.0±0.25**

Each value is the mean ± SEM for 5mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

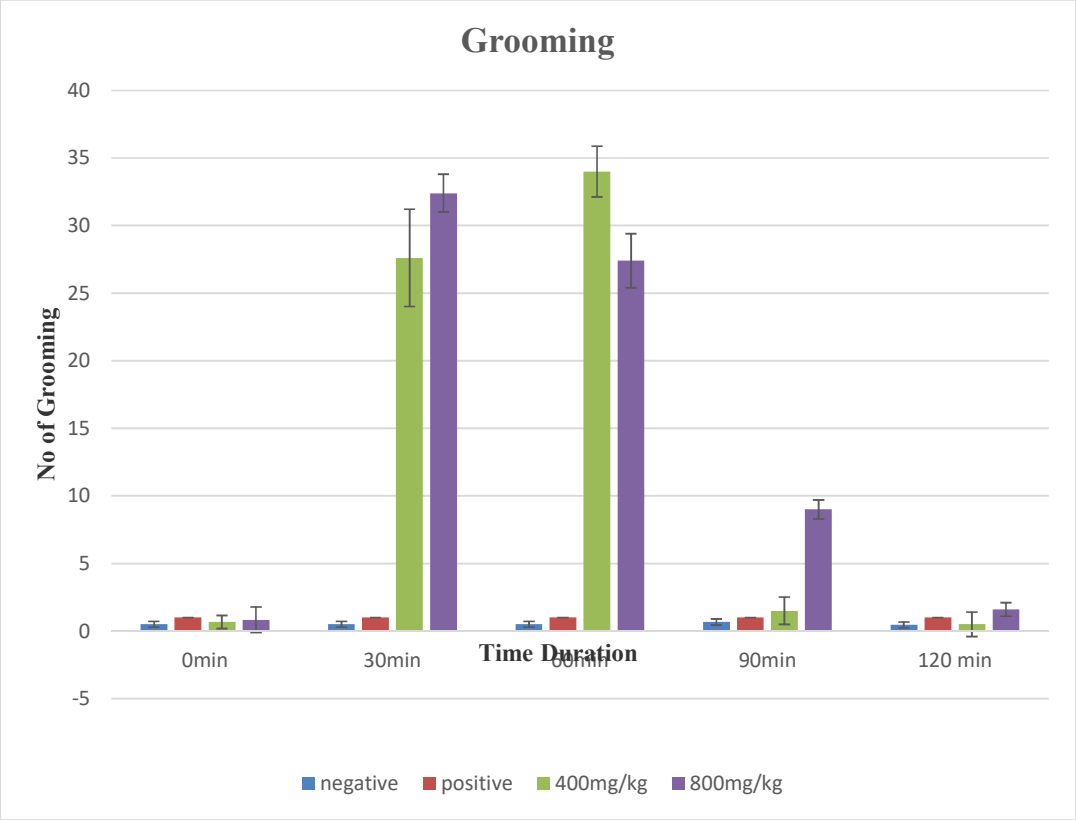


Figure-40: Graphical Presentation of CNS Activity of plant extract of *Drynaria quercifolia* by Open Field Test (Grooming) in Mice

Table41: CNS Activity of plant extract of *Drynaria quercifolia* 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	20.33±0.76***	21.16±0.13***	21.33±0.80***	17.67±0.76***	21.33±0.80***
Crude extract of	400mg/kg	16.2±0.86***	4.8±0.66***	26.0±0.67***	.20±0.2***	.20±0.2***
Crude extract of	800mg/kg	13.0±1.2***	2.6±0.73***	.20±0.2***	.20±0.2***	0.0±0.0
Positive control, Diazepam	1mg/kg	18.33±0.42***	9.0±0.57***	7.5±0.42***	5.0±0.36***	2.83±0.54***

Each value is the mean ± SEM for 5mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test.

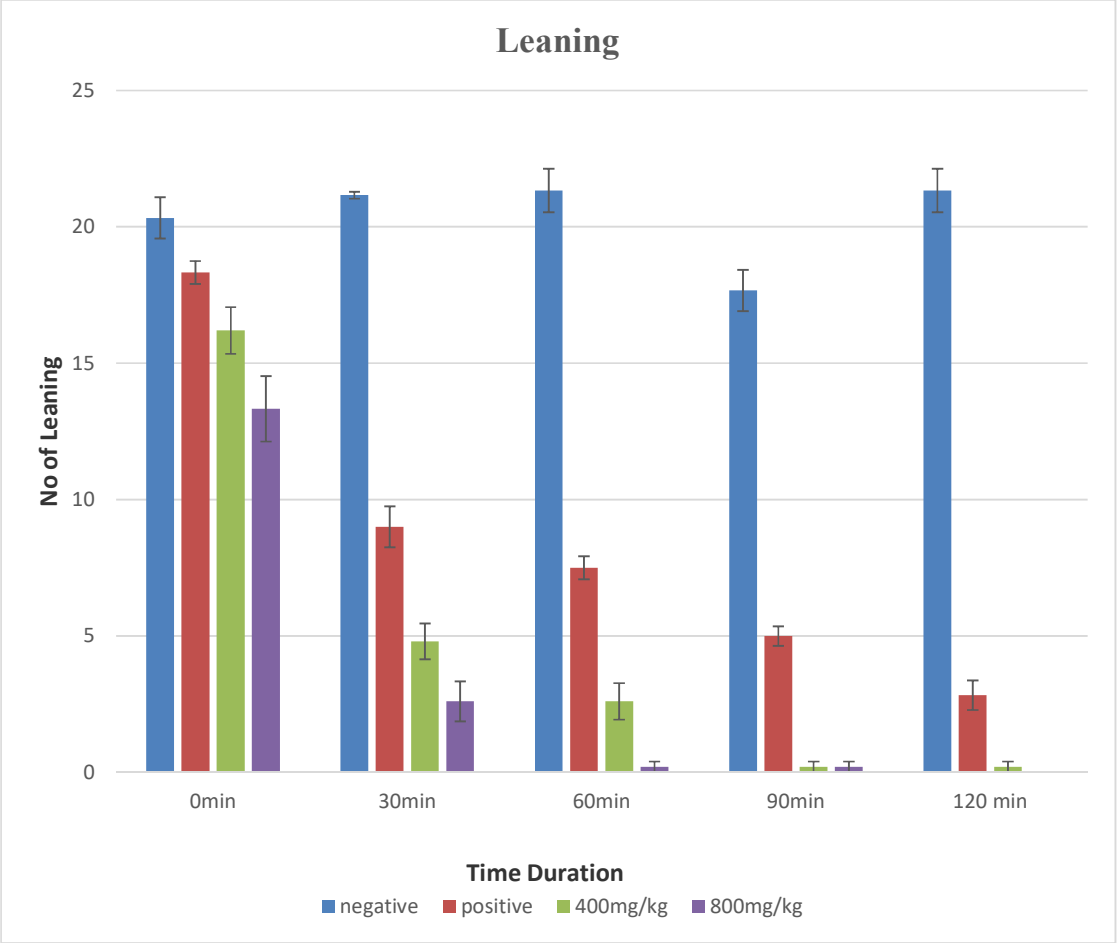


Figure-42: Graphical Presentation of CNS Activity of plant extract of *Drynaria quercifolia* by Open Field Test (Leaning) in Mice.

4.2.2.1Hole Board Test:

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

Total Number of Head Poking and Head Dipping count

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

This group of animals only received vehicle (5% CMC) 10 ml/kg orally. The observed total number of head poking is with a mean value of 54.33±0.88 (Mean ±SEM) and head dipping with mean value of (Mean ±SEM) during 5 minutes observation after 30 min of administration..

Test Group-1 (Plant Extract, 400mg/kg)

This test group of mice receive the plant extract of 200 mg/kg orally. The observed total number of head poking is with a mean value of 11.2±0.58 (Mean ±SEM) and head dipping with mean value of 6.8±0.68 (Mean ±SEM) during 5 minutes observation after 30 min of administration.

Test Group-2 (Plant Extract, 800mg/kg)

This group of mice receive the plant extract of 400 mg/kg orally. The observed total number of head poking is with a mean value of 38.8±0.21 (Mean ±SEM) and head dipping with mean value of 18.8±1.2 (Mean ±SEM) during 5 minutes observation after 30 min of administration.

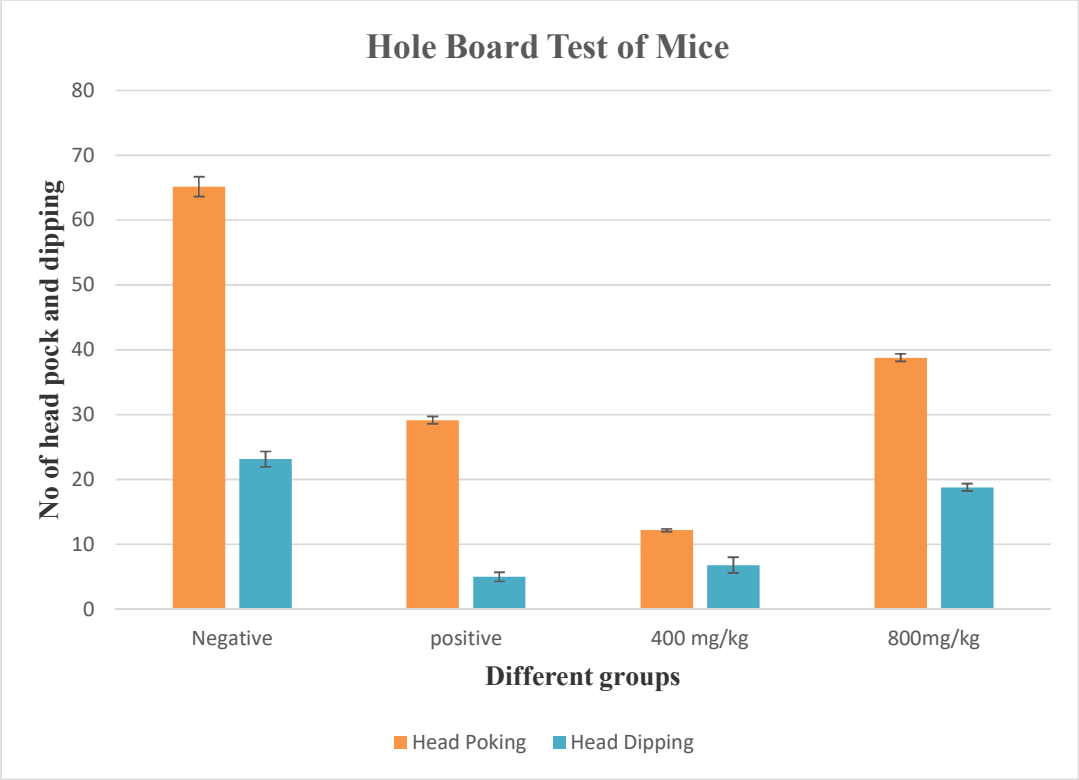
Positive Control Group (Indomethacin, 10mg/kg)

This group of mice receives the standard drug Diazepam of 1mg/kg orally. The observed total number of head poking is with a mean value of 54.33±0.88 (Mean ±SEM) and head dipping with mean value of (Mean ±SEM) during 5 minutes observation after 30 min of administration.

Table–12: CNS Activity of plant extract of *Drynaria quercifolia* by Hole Board Test in Mice.

Groups	Treatment	Dose	No. of Head Poking	No. of Head Dipping
Negative control	5% CMC	10ml/kg	65.16±1.55***	23.16±1.19***
Group-1	Crude extract of ***Drynaria quercifolia***	400mg/kg	11.2±0.58***	6.8±0.68***
Group-2	Crude extract of ***Drynaria quercifolia***	800mg/kg	38.8±0.21***	18.8±1.2***
Positive control	Diazepam	1mg/kg	29.16±0.60***	5.0±0.57***

Each value is the mean ± SEM for 6 mice , * P < 0.5; ** P < 0.01; *** P < 0.001 compared with control. Data were analyzed by using One-way ANOVA followed by Dunnett's test



Figure–43: Graphical Presentation of CNS Activity of plant extract of *Drynaria quercifolia* by Hole Board Test in Mice.

4.3. Acute and Sub Chronic Toxicity Test

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

4.3.2. Sub Chronic Toxicity Test:

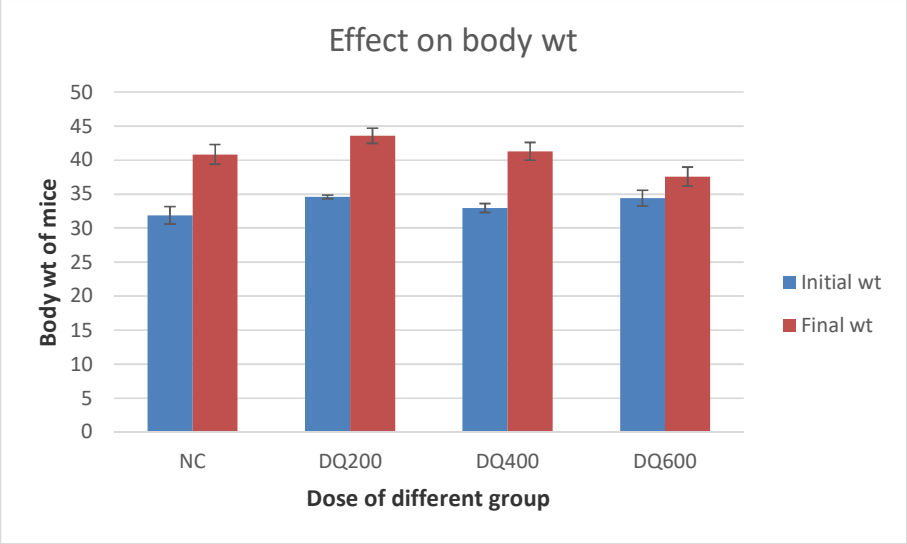
4.3.2.1. CBC (Count Blood Cell) Test, Biochemical Test &Histological Studies

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

In the subchronic study of methanolic extract of *Drynaria quercifolia*at a dose (200,400,600 mg/kg) to the mice, significant difference were found in the erythrocyte and leucocytes values of both the treated and control mice. In which case, the administration of *Drynaria quercifolia*methanol extract for a period of 45 days induce significant anaemia. Also some irregularities were observed mainly in the RBC, WBC, Platelet and SGPT (hepatic enzymatic test). This could be as a result of the mice response to foreign bodies associated with the chronic toxicity during the experiment.In sub-chronic study, we observed significant decrease in body weights than *Drynaria quercifolia*treated group (after 45 days) from control group. The toxicity assay also result some abnormality and mortality of the tested mice for the period of 45 days monitored. At the end of the study (after 45 days) 3 mice shows abnormalities and overall 11 mice died in 3 doses of groups.

Table-13: Effect of methanolic extract of *Drynaria quercifolia*on body weight in mice

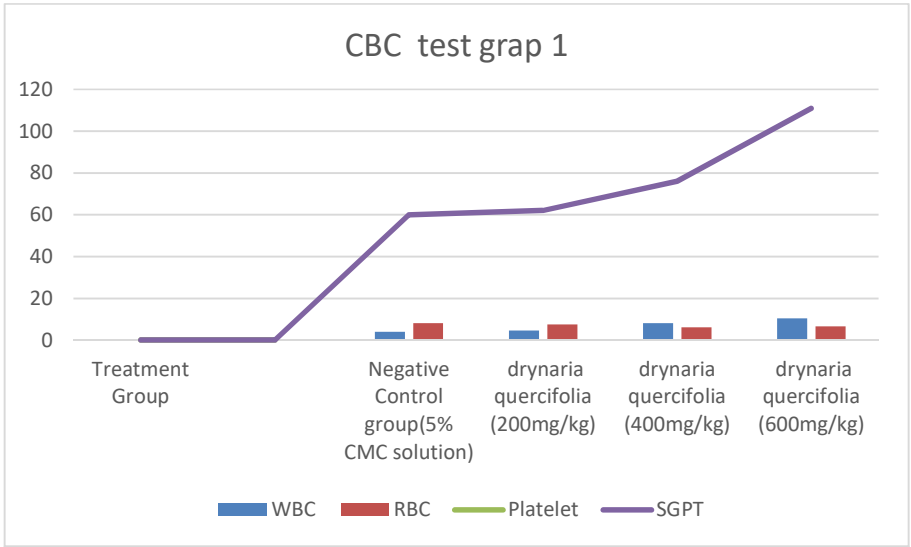
Treatment Groups	Gender	Initial body weight	Final body weight	No. of death
Normal Control (5% CMC)	Male	31.89±1.28	40.84±1.43	3
Drynaria quercifolia 200mg/kg	Male	34.58±0.25	43.58±1.1	3
Drynaria quercifolia 400mg/kg	Male	32.95±0.67	41.28±1.3	3
Drynaria quercifolia 600mg/kg	Female	34.40±1.14	37.57±1.4	2

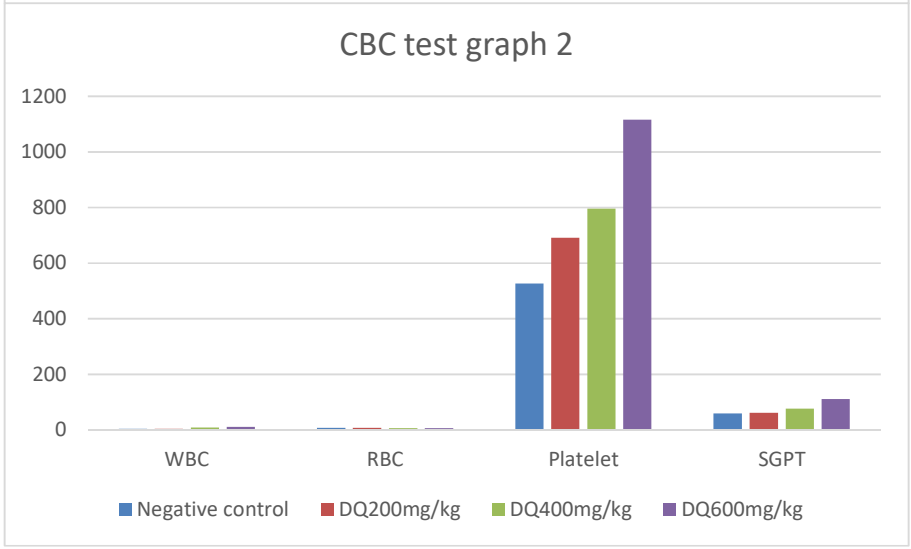


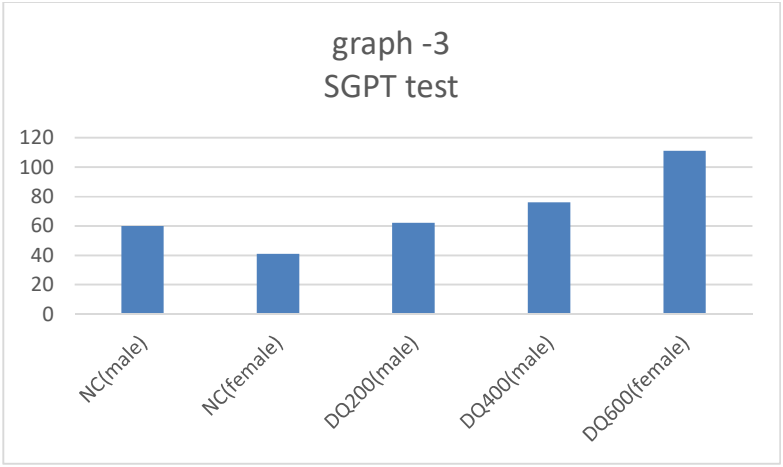
Figure–44 Effect of methanolic extract of *Drynaria quercifolia*on body weight in mice

Table14: Effect of *Drynaria quercifolia* on the CBC (Count Blood Cell) &Biochemical Test

Treatment Group	WBC $10^3/mm^3$ (n)	RBC $10^6/mm^3$ (n)	Platelet $10^3/mm^3$ (n)	SGPT (IU/dl) (n)
Negative Control group(5% CMC solution)	4.07	8.08	1274	60 (Male) 41 (Female)
drynaria quercifolia (200mg/kg)	4.67	7.49	692	62
drynaria quercifolia (400mg/kg)	8.2	6.1	796	76
drynaria quercifolia (600mg/kg)	10.5	6.7	1115	111







Figures 45: CBC (Count Blood Cell) & Biochemical Test of Drynaria

Table15: Effect of *Drynaria quercifolia* on the Different count of WBC (White Blood Cell)

Treatment Group	Total WBC	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Negative Control group(5% CMC solution)	4.07	13.9	82.2	8.25	19.65	6.96
***Drynaria quercifolia* (200mg/kg)**	4.67	28	57	26	30	4
***Drynaria quercifolia* (400mg/kg)**	8.2	16.3	54.4	2.7	24.5	2
***Drynaria quercifolia* (600mg/kg)**	10.5	12	78	4	4	2

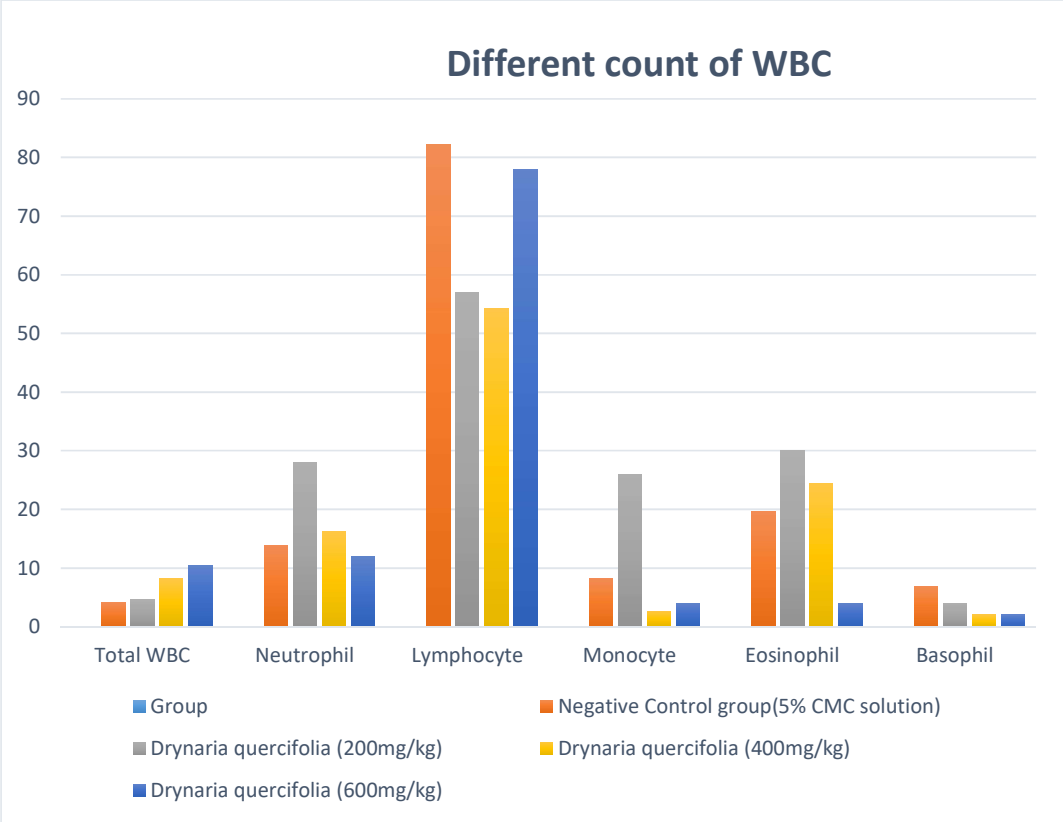


Figure 46: Effect of *Drynaria quercifolia* on the Different count of WBC (White Blood Cell)

Table 16: Effect of *Drynaria quercifolia* on the Different count of RBC (Red Blood Cell)

Treatment Group	Total RBC	Haemoglobin	Hematocrit (HCT)	Mean Corpuscular Volume (MCV)	Mean Corpuscular Haemoglobin (MCH)	Mean Corpuscular Hematocrit Cell (MCHC)	Red blood cell distribution width (RDW)
Negative Control group(5% CMC solution)	8.08	13.9	67.17	85.8	16.35	19	19.37
***Drynaria quercifolia* (200mg/k)**	7.49	11.9	65.8	87.8	15.9	18.1	18.3
***Drynaria quercifolia (*400mg/k)**	6.1	11.7	58.9	97.2	19.2	19.4	20.2
***Drynaria quercifolia* (600mg/kg)**	6.7	14	53.8	79.4	20.7	26	17

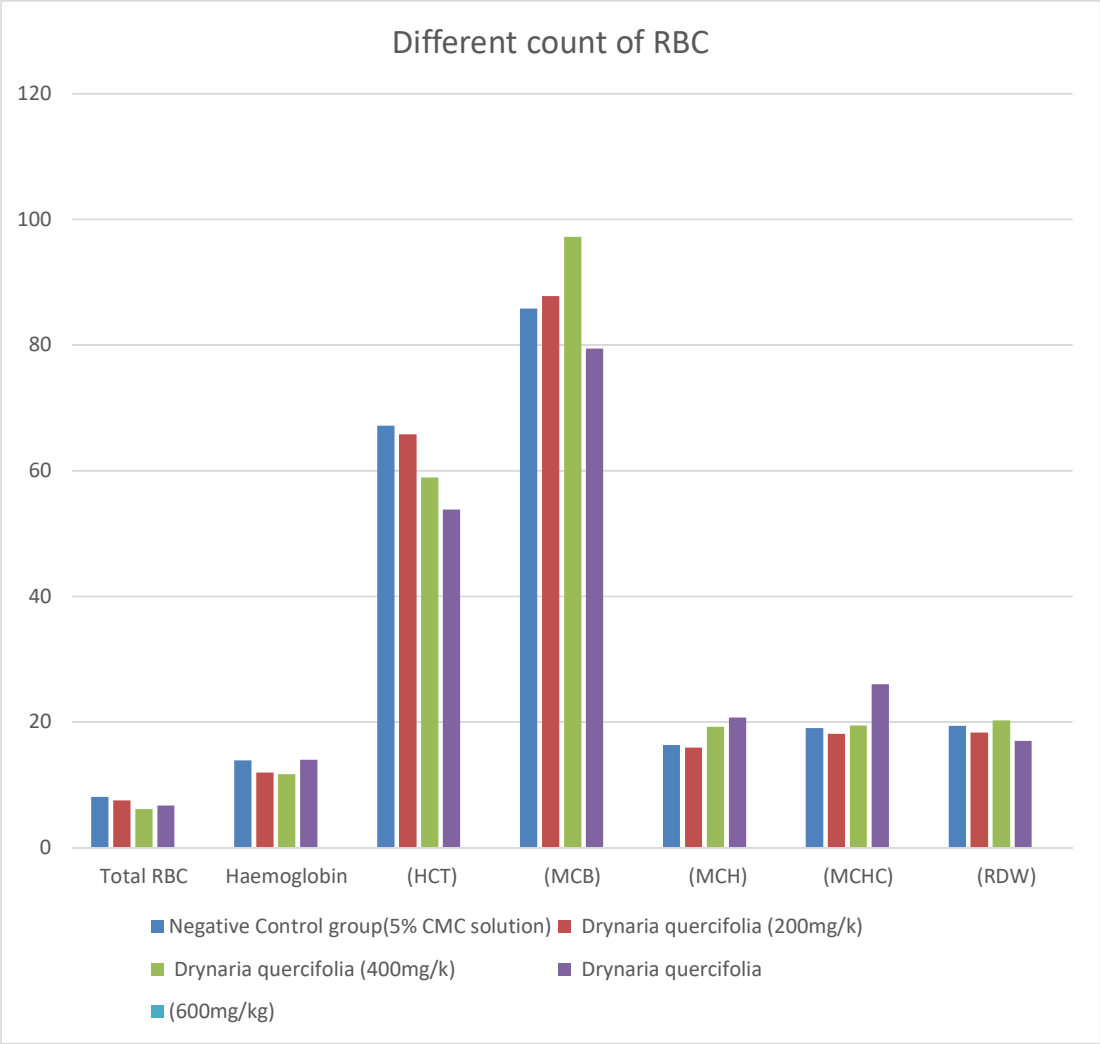


Figure47: Effect of *Drynaria quercifolia* on the Different count of RBC (Red Blood Cell)

4.3.2.2Histopathological studies

The histological status of the liver tissues of both the treated and control mice where normal cellular architecture with prominent central vain was shown which indicates, that the extract can cause damage to liver if used for therapeutic purpose. This becomes important because liver is the primary organ for detoxification. The criticism in traditional medicine is the lack of scientific evaluations to justify its tremendous impact in its use as a safe drug.

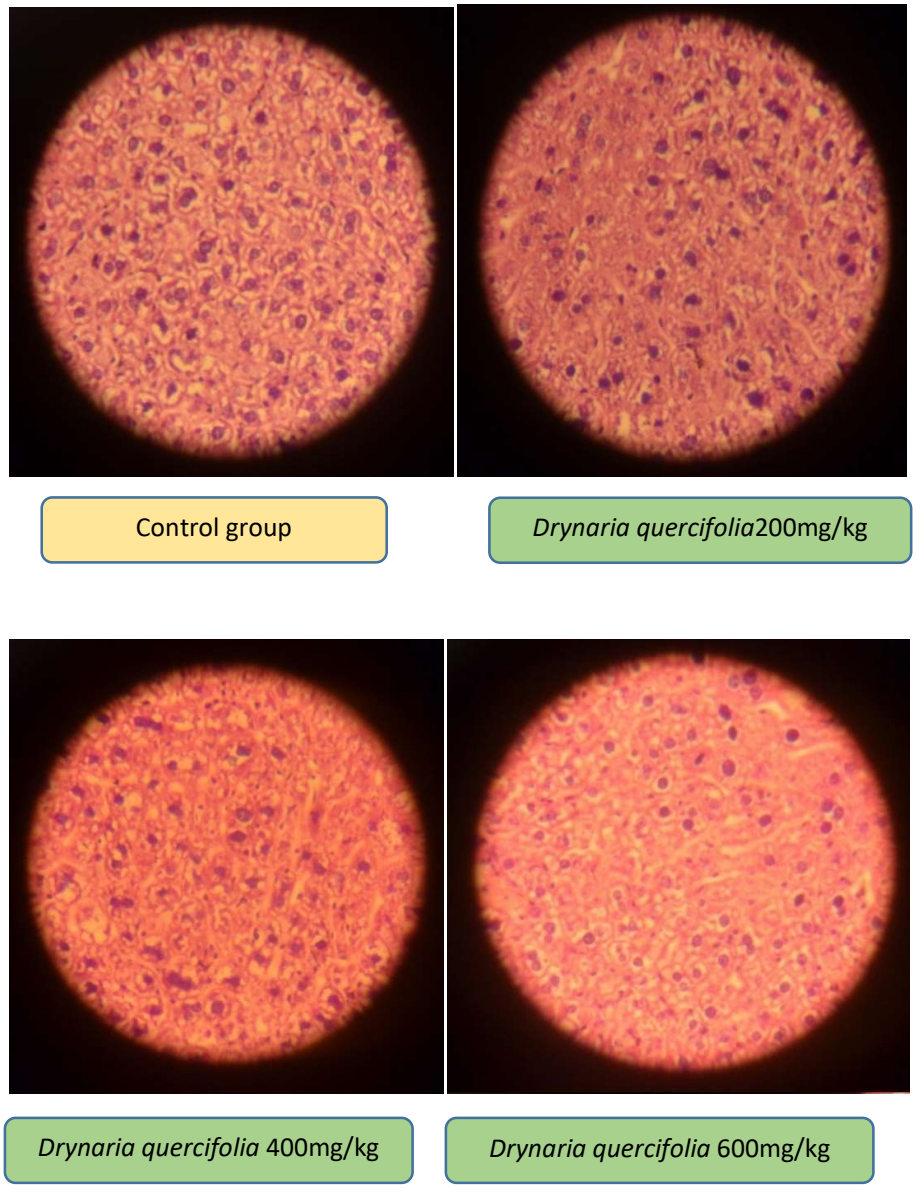


Figure-48: Histopathological test of mice in different group

5 Conclusion:

Our results revealed that the fraction (n-hexen and dichloromethane) of metanolic extract of *Drynaria quercifolia* appears to contain substance(s) that possess significant Analgesic activity.

The result of the effect of the plant crude extract against acetic acid induced writhing in mice is shown in Table-. The crude extract (200mg/kg)dose dependently reduces the number of abdominal constriction and stretching of hind limb induced by the injection of acetic acid.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, Indomethacin 10mg/kg .The reference drug is found slightly potent than the extract.The percent inhibition of writhing by groups pretreated with *Drynaria quercifolia* (200mg/kg)and Indomethacin is almost similar,

In addition *Drynaria quercifolia* has also has medicinal value on psychological aspect that shown (400mg\kg and 800mg\kg)potent depressive and anxiolytic effect which need further investigation.The crude extract (400mg/kg and 800mg\kg)dose show significant result on hole board and 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, diagepam1mg/kg.

The aim of this toxicological study was to investigate to found the possible toxicity of the plant *Drynaria quercifolia*. For finding sub-chronic toxicity several tests are done such as CBC (Cell Blood count) test, Hepatic enzyme test and histological Studies. CBC test and hepatic enzyme test are done by hematological machine and histological 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 *Drynaria quercifolia* extract which significant difference were not found in RBC but difference were found in WBC & different count of WBC (Neutrophil, Monocyte, Eosinophil,& Basophil) values of both treated & control mice. The result shows that increase toxic effect by increase dose such as (200,400 & 600) mg/kg and decrease (Neutrophil, Monocyte, Eosinophil,& Basophil) values and also increase SGPT value. The histopathological status of the liver tissues of both the treated mice where normal

cellular architecture with prominent central vain was shown which indicates that the extract can cause damage to liver if used for maximum dose.

The larvicidal test also show moderately promising result.

Results of our pharmacological study suggest the great value of the species *Drynaria quercifolia* for use inpharmacy but its toxicological study recommend to use it consciously.

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Annexure

List of Abbreviation	Full Meaning
AAS	Anabolic Androgenic Steroid
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
COX	Cyclo-oxygenase
DCM	Dichloromethane
DMPP	Descending Modulatory Pain Pathways
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetra Acetic acid
EMG	Electromyography
EP	Evoked Potential
EVF	Erythrocyte Volume Fraction
GGT	Gamma-Glutamyl Transferase
GPT	Glutamate Pyruvate Transaminase
HCT	Hematocrit
HIV/AIDS	Human Immunodeficiency Virus Infection and Acquired Immune Deficiency Syndrome
IASP	International Association for the Study of Pain
ICDDR, B	International Centre for Diarrhoeal Disease and Research, Bangladesh
LFTs or LFs	Liver Function Tests
LOX	Lipoxygenase

MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Cell Volume
MMPI	Minnesota Multiphasic Personality Inventory
MRIs	Magnetic Resonance Imaging's
MS	Multiple sclerosis
NCCAM	National Center for Complementary & Alternative Medicine
NSAID	Non-Steroidal Anti-inflammatory Drug
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
UDA	Undeca-2*E*-ene-8,10-Diynoic acid Isobutylamide
WBC	White Blood Cell
5-HT	5-hydroxytryptamine