Pharmacological and Toxicological Study of Methanolic Extract of *Curcuma Caesia* with the evaluation of Antioxidant and Cytotoxic Activity of *Curcuma Caesia*

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A thesis report, submitted to the Department of Pharmacy, East West University, in partial fulfillment of the requirements for the degree of Bachelor of Pharmacy.

CERTIFICATE BY THE SUPERVISOR

This is to certify that the dissertation entitled "**Pharmacological and Toxicological Study of Methanolic Extract of *Curcuma Caesia* with the evaluation of Antioxidant and Cytotoxic Activity of *Curcuma Caesia***" is a bona-fide research work done by Farzana Haque Mishu **,** ID: 2013-1-70-017 in partial fulfillment of the requirement for the Degree of Bachelor of Pharmacy.

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DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled "**Pharmacological and Toxicological Study of Methanolic Extract of *Curcuma Caesia* with the evaluation of Antioxidant and Cytotoxic Activity of *Curcuma Caesia***" is an authentic and genuine research work carried out by me under the guidance of **Dr. Shamsun Nahar Khan,** Associate Professor, Department of Pharmacy, East West University, Dhaka, Bangladesh.

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CERTIFICATE

This is to certify that, the research work on "**Pharmacological and Toxicological Study of Methanolic Extract of *Curcuma Caesia* with The Evaluation of Antioxidant and Cytotoxic Activity** " submitted to the department of pharmacy, East West University, Dhaka, Bangladesh, in partial fulfillment of the requirement for the degree of Bachelor of pharmacy (B.Pharm) was carried out by Farzana Haque Mishu**,** ID: 2013-1-70-017, 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 resources of the information in this connection are duly acknowledged.

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Introduction

ABSTRACT

Purpose: The research work was carried out to determine the pharmacological activities and toxicology of methanolic extract of Curcuma Caesia. Evaluation of Antioxidant and Cytotoxic Activity of *Curcuma Caesia.*

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

Result: The plant was found to have depressive and toxic effect. The CNS activity was evaluated by open field method. In the open field method and hole board experiment the crude extract of Curcuma Caesia (200mg/kg, 400mg/kg & 800mg/kg) dose dependently reduces the number of peripheral locomotion, central locomotion and leaning in the open field test and reduces the number of head dipping and head poking in the hole board test. The reduction is significant (***$p<0.001$) when it is compared to the standard drug. For finding chronic toxicity several tests are done such as CBC (Cell Blood count) test, Hepatic enzyme test and histopathological Studies. All data were analyzed by using SPSS analytical method.

Conclusion: After summarize all the results it can say that bark of Curcuma Caesia may have several pharmacological activities. *Curcuma Caesia* was screened for their antimicrobial and antioxidant activities. The part which was used in the research study was methanolic extract of leaves. Methanolic extracts of *Curcuma Caesia* leaves exhibited strong antioxidant and antibacterial activities. First phyto-chemical test was done where methanolic extract of leaves showed high content of flavonoid & tannins. DPPH scavenging activity test & total phenolic content test is rendered then to monitor the anti-oxidant activity. In DPPH scavenging activity test The IC_{50} or inhibitory concentration of the sample was obtained (28.30 µg/ml) much more higher than the standard (Ascorbic acid) compound. Methanolic extract of leaves also showed high phenoilic content (223.1333±24.5375 mg/g).

Keywords: *Curcuma Caesia,* CNS, Neuro-pharmacological effect and Toxicity test.

1.1 Medicinal Plant

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

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

1.1.1 Definitions of medicinal plants

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

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

1.1.2 Importance of Medicinal Plant

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

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

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

Based on their biosynthetic origins, plant natural products can be divided into three major groups: the terpenoids, the alkaloids, and the phenolic compounds. All terpenoids, including both primary metabolites and more than 25,000 secondary compounds, are derived from the five-carbon precursor isopentenyl diphosphate (IPP). The 12,000 or so known alkaloids, which

contain one or more nitrogen atoms, are biosynthesized principally from amino acids. The 8000 or so phenolic compounds are formed by way of either the shikimic acid pathway or the malonate/acetate pathway (Ghani, 2003).

1.1.2 Medicinal plants & Traditional Medicine Practice in Bangladesh

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

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

1.2 Pain

Pain is a sensorial modality and primarily protective in nature, but often causes discomfort. It is the most important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause (Mate, G.S, 2008). Currently available analgesic drugs such as opiates and NSAIDs are not useful in all cases due to their adverse effects. In this respect new compounds with improved pain management capacity and fewer side effects are being sought with urgency (Halliwell, B, 1990).

1.2.1 Definition & Types of Pain

Pain is a universal human experience. The International Association for the Study of Pain (IASP) defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage." Pain may be a symptom of an underlying disease or disorder, or a disorder in its own right.

At the same time that pain is a universal experience, however, it is also a complex one. While the physical sensations involved in pain may be constant throughout history, the ways in which humans express and treat pain are shaped by their respective cultures and societies. Since the 1980s, research in the neurobiology of pain has been accompanied by studies of the psychological and sociocultural factors that influence people's experience of pain, their use of health care systems, and their compliance with various treatments for pain. As of 2003, the World Health Organization (WHO) emphasizes the importance of an interdisciplinary approach to pain treatment that takes this complexity into account (Rebecca J. Frey, 2004).

Pain can be classified by different way:

1.2.2 Classification of pain:

The four most commonly used systems 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) 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 somatic and visceral pain depending on the location of activated nociceptors.

• **Somatic Pain:**

Somatic pain is 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 pain is 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 pain is 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.

2) 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 chronic pain 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.

3) 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.

4) Anatomical 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.

5) 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)

1.2.3 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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1.2.3.1 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.

1.2.3.2 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-1: Characteristics and Functions of C fibre and A-delta fibres.

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

1.2.3.3 Transmission 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.

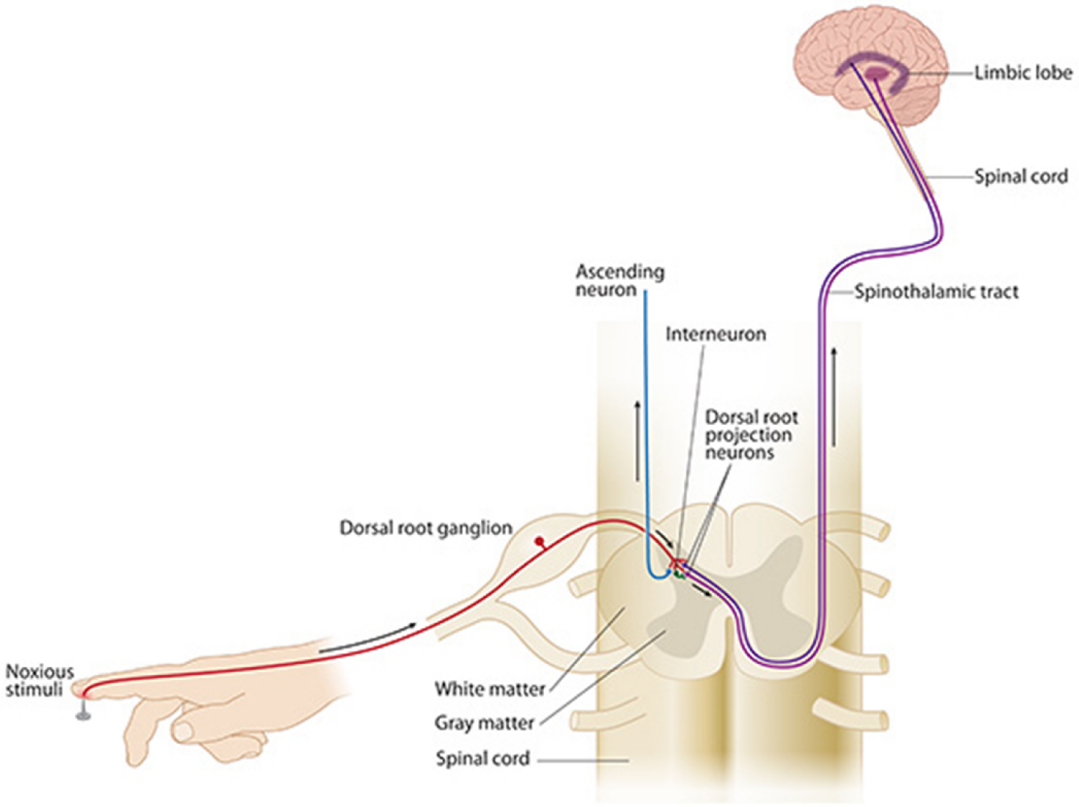


Figure 1.1: Transmission of Pain (Wood, 2008)

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.

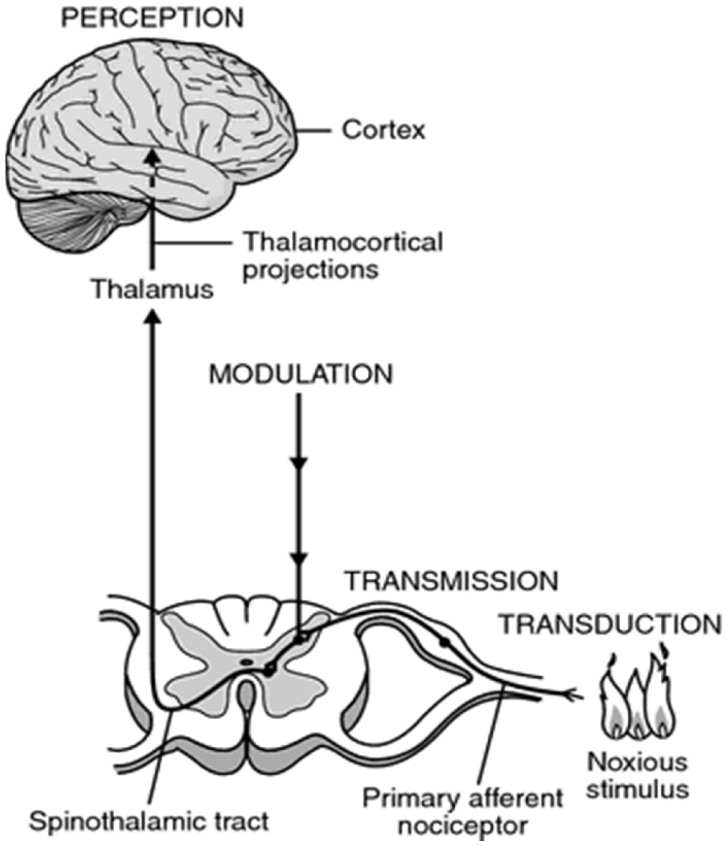


Figure-1.2: Pain Pathway and Mechanism. (Wood, 2008)

1.2.3.4 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).

1.2.3.5 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)

1.2.4 Evaluation of pain

Patient description and 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. The most important part of pain management is removing the underlying cause(s) whenever possible, even when there is a psychological component to 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 cause(s) 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).

1.2.4.1 Factors that affect pain perception

Location and severity of pain:

Pain varies in intensity and quality. It may be mild, moderate, or severe. In terms of quality, it may vary from a dull ache to sharp, piercing, burning, pulsating, tingling, or throbbing sensations; for example, the pain from jabbing one's finger on a needle feels different from the pain of touching a hot iron, even though both injuries involve the same part of the body. If the pain is severe, the nerve cells in the dorsal horn transmit the message rapidly; if the pain is relatively mild, the signals are transmitted along a different set of nerve fibers at a slower rate.

Gender:

Recent research has shown that sex hormones in mammals affect the level of tolerance for pain. The male sex hormone, testosterone, appears to raise the pain threshold in experimental animals, while the female hormone, estrogen, appears to increase the animal's recognition of pain. Humans, however, are influenced by their personal histories and cultures as well as by body chemistry.

Studies of adult volunteers indicate that women tend to recover from pain more quickly than men, cope more effectively with it, and are less likely to allow pain to control their lives.

Family:

Another factor that influences pain perception in humans is family upbringing. Some parents comfort children who are hurting, while others ignore or even punish them for crying or expressing pain. Some families allow female members to express pain but expect males to "keep a stiff upper lip." People who suffer from chronic pain as adults may be helped by recalling their family's spoken and unspoken "messages" about pain, and working to consciously change those messages.

Culture and ethnicity:

In addition to the nuclear family, a person's cultural or ethnic background can shape his or her perception of pain. People who have been exposed through their education to Western explanations of and treatments for pain may seek mainstream medical treatment more readily than those who have been taught to regard hospitals as places to die. On the other hand, Western medicine has been slower than Eastern and Native American systems of healing to recognize the importance of emotions and spirituality in treating pain. There are also differences among various ethnic groups within Western societies regarding ways of coping with pain. One study of African American, Irish, Italian, Jewish, and Puerto Rican patients being treated for chronic facial pain found differences among the groups in the intensity of emotional reactions to the pain and the extent to which the pain was allowed to interfere with daily functioning (Rebecca J. Frey, 2004).

1.2.6 Treatment of pain

Treatment of either acute or chronic pain may involve several different approaches to therapy.

Medications

Medications to relieve pain are known as analgesics. Aspirin and other nonsteroidal anti-inflammatory drugs, or NSAIDs, are commonly used analgesics. NSAIDs include such

medications as ibuprofen, ketoprofen, diclofenac, naproxen, and nabumetone. These medications are effective in treating mild or moderate pain. 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.

For more severe pain, the doctor may prescribe an NSAID combined with an opioid, usually codeine or hydrocodone. Opioids, which are also called narcotics, are strong painkillers derived either from the opium poppy *Papaver somniferum* or from synthetic compounds that have similar effects. Opioids include such drugs as codeine, fentanyl, hydromorphone, meperidine, morphine, oxycodone, and propoxyphene. They are defined as Schedule II controlled substances by the Controlled Substances Act of 1970.In addition to the risk of abuse; opioids cause potentially serious side effects in some patients, including cognitive impairment (more common in the elderly), disorientation, constipation, nausea, heavy sweating, and skin rashes.

Surgery

Because surgery is itself a cause of pain, few surgical treatments to relieve pain were available prior to the discovery of safe general anesthetics in the mid-nineteenth century. For most of human history, doctors were limited to procedures that could be completed within two to three minutes because the patients could not bear the pain of the operation. Ancient Egyptian doctors gave their patients wine mixed with opium, while early European doctors made their patients drunk with brandy, tied them to the benches that served as operating tables, or put pressure on a nerve or artery to numb a specific part of the body.

Modern surgeons, however, can perform a variety of procedures to relieve either acute or chronic pain, depending on its cause. These procedures include:

- removal of diseased or dead tissue to prevent infection
- removal of cancerous tissue to prevent the spread of the cancer and relieve pressure on nearby healthy organs and tissues
- correction or reconstruction of malformed or damaged bones
- insertion of artificial joints or other body parts to replace damaged structures

- organ transplantation
- insertion of pacemakers and other electrical devices that improve the functioning of damaged organs or help to control pain directly
- cutting or destroying damaged nerves to control neuropathic pain

Psychotherapy

Psychotherapy may be helpful to patients with chronic pain syndromes by exploring the connections between anger, depression, or anxiety and physical pain sensations. One type of psychotherapy that has been shown to be effective is cognitive restructuring, an approach that teaches people to "reframe" the problems in their lives—that is, to change their conscious attitudes and responses to these stressors. Some psychotherapists teach relaxation techniques, biofeedback, or other approaches to stress management as well as cognitive restructuring. Another type of psychotherapy that is effective in treating some patients with chronic pain is hypnosis.

Complementary and 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 and 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 Definition of Anti-inflammatory effect

Anti-inflammatory refers to the property of a substance or treatment that reduces inflammation. Anti-inflammatory drugs make up about half of analgesics, remedying pain by reducing inflammation as opposed to opioids which affect the central nervous system.

Anti-inflammatory painkillers are sometimes called non-steroidal anti-inflammatory drugs (NSAIDs), or just 'anti-inflammatories'. There are over 20 types. Anti-inflammatories are used to ease pain in various conditions including: arthritis (various types), muscle and ligament pains (strains and sprains), period pain, pains after operations, headaches, migraines, and some other types of pain. Ibuprofen and aspirin are also used to bring down a high temperature. Low dose aspirin is also used to help prevent blood clots that can cause a heart attack or stroke (Patient UK, 2006).

1.3.1 Non-steroidal anti-inflammatory drugs (NSAID)

NSAIDs -- or nonsteroidal anti-inflammatory drugs -- are among the most common pain relief medicines in the world. Every day more than 30 million Americans use them to soothe headaches, sprains, arthritis symptoms, and other daily discomforts, according to the American Gastroenterological Association (AGA). And as if that wasn't enough, in addition to dulling pain NSAIDs also lower fever and reduce swelling (R. Morgan Griffin, 2010).

1.3.2 Chemistry & Pharmacokinetic of NSAID

There many differences in the kinetics of NSAIDs, they have some general properties in common. All but one of the NSAIDs are weak organic acids as given the exception, nabumetone, is a ketone prodrug that is metabolized to the acidic active drug. Most of these drugs are well

absorbed, and food does not substantially change their bioavailability. Most of the NSAIDs are highly metabolized, some y phase I & phaseII mechanisms & othersby direct glucuronides alone. Metabolism of most NSAIDs proceeds, in part, by way of the CYP3A or CYP2C families of P450 enzymes in the liver. While renal excretion is the most important route for final elimination, nearly all undergo varying degrees of biliary excretion & reabsorption. Infact the degree of lower gastrointestinal tract irritation correlates with the amount of enterohepatic circulation. Most of the NSAIDs are highly protein bound, usually to albumin. Some of the NSAIDs are racemic mixture (eg. Ibuprofen), while one , naproxen is provided as a single enantiomer, and a few have no chiral center (eg. Diclofenac) (Bertram G. Katzung, 2001).

1.3.3 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 **(Cashman JN, 1996)**

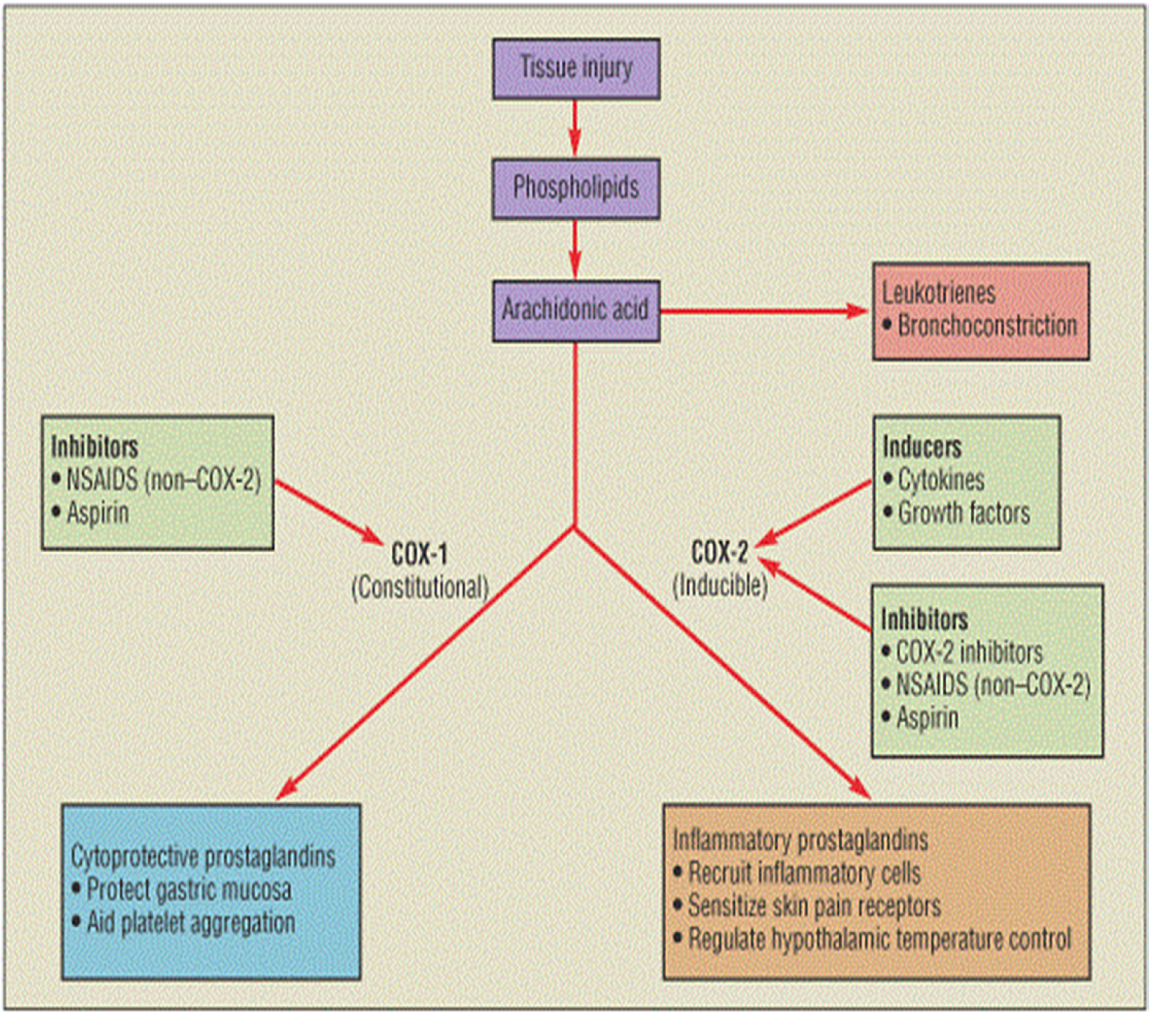


Figure 1.3: Pathway of inhibition of NSAIDs (Cashman JN, 1996)

1.3.3 Indications of NSAID

NSAIDs can be used as simple pain killers (analgesics), but paracetamol is usually preferable, as it is likely to have less unwanted effects and costs less. They are most useful in conditions which cause inflammation. The anti-inflammatory effects may take from a few days to three weeks to come on, so it is worth persevering for a while before deciding that a NSAID is not going to help.

1.3.4 Uses of NSAIDs

- The commonest use of these drugs is for arthritis. Paracetamol is often adequate for osteoarthritis, but NSAIDs are particularly useful in the inflammatory forms of arthritis (eg rheumatoid arthritis) and, sometimes, in the more severe forms of osteoarthritis.
- Back pain and sciatica. Ibuprofen has been clearly demonstrated to be helpful, and the other NSAIDs are also helpful.
- Sprains, strains, and rheumatism.
- Dental pain.
- Post-operative pain.
- Period pain (dysmenorrhoea) and heavy periods (menorrhagia).
- Pain from kidney stones (renal colic).
- To help reduce temperature in someone with a fever.
- Migraine.
- Other painful conditions, especially where there is inflammation.
- A recent Dutch study suggested that regular and long-term use of some NSAIDs could reduce the risk of Alzheimer's disease by as much as 80 percent. Note that this is only one study.
- Most NSAIDs also reduce the temperature in someone with a fever (USGyms.Net, 2010).

1.3.5 Types of Nonsteroidal Anti-inflammatory drugs

There are two main types of NSAIDs, nonselective and selective. The terms nonselective and selective refer to different NSAIDs ability to inhibit specific types of cyclooxygenase (COX) enzymes.

- Nonselective NSAIDs — Nonselective NSAIDs inhibit both COX-1 and COX-2 enzymes to a similar degree. Nonselective NSAIDs include commonly available drugs such as aspirin, ibuprofen , and naproxen.
- Selective NSAIDs — Selective NSAIDs inhibit COX enzymes found at sites of inflammation (COX-2) more than the type that is normally found in the stomach, blood platelets, and blood vessels (COX-1). Selective NSAIDs (also called COX-2 inhibitors)

are as effective in relieving pain and inflammation as nonselective NSAIDs and are less likely to cause gastrointestinal injury. Celecoxib is the only selective NSAID currently available in the United States. Selective NSAIDs are sometimes recommended for people who have had a peptic ulcer, gastrointestinal bleeding, or gastrointestinal upset when taking nonselective NSAIDs. Selective NSAIDs have less potential to cause ulcers or gastrointestinal bleeding, but they do not prevent ulcers that develop for other reasons (Daniel H Solomon, 2010).

1.3.6 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).

NSAIDs cannot be used in the following cases:

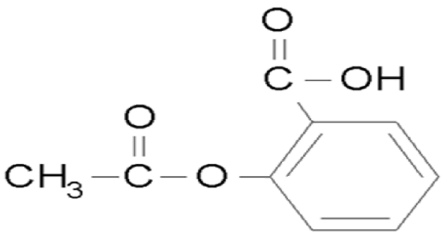
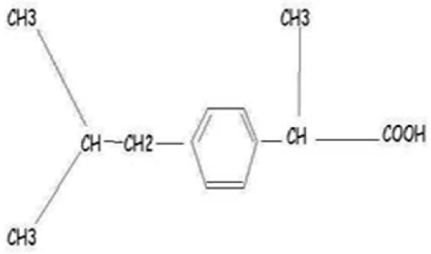
- When patient has an allergy to aspirin or any NSAID
- During pregnancy
- During breast feeding
- While patient is on blood thinning agents (anticoagulants)
- Suffering from a defect of the blood clotting system (coagulation)
- While patient has an active peptic ulcer
- While patient is being treated for a fracture use should be monitored.

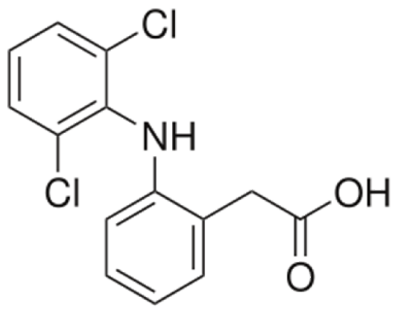
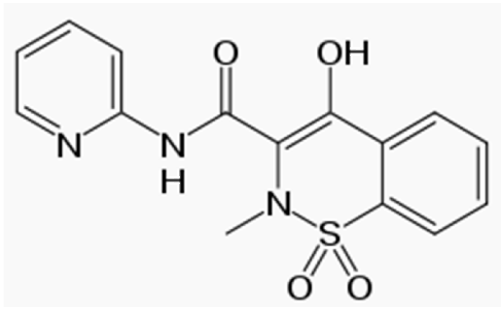
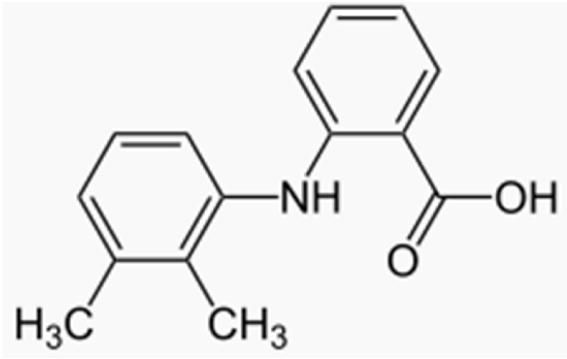
Care is needed if one has:

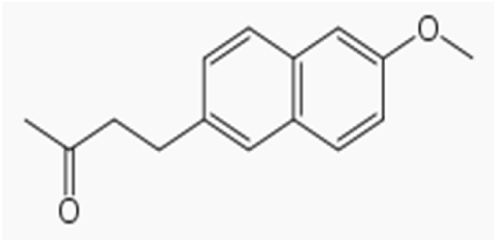
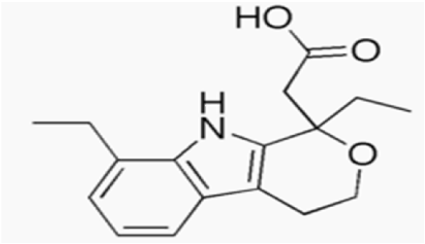
- Asthma
- Liver impairment
- Heart impairment
- Kidney impairment (USGyms.Net, 2010) (Fries James F., 1991)

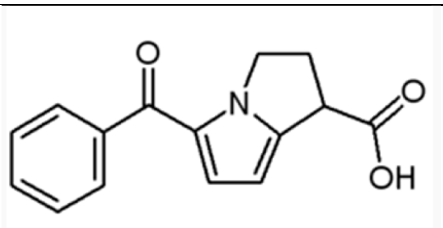
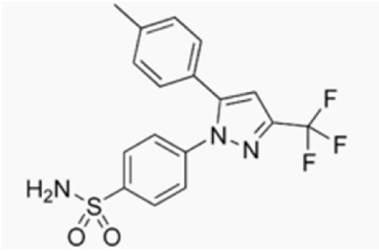
1.3.7 Classification of NSAIDs

Table 2: Different types of NSAIDs

Salicylic acids	Aspirin (acetylsalicylic acid) , Choline magnesium trisalicylate, Diflunisal, Salsalate  Structure of Aspirin
Propionic acids	Fenoprofen, Flurbiprofen, Ibuprofen, Ketoprofen, Naproxen, Oxaprozin  Structure of Ibuprofen

Acetic acids	Diclofenac, Indomethacin, Sulindac, Tolmetin  Structure of Diclofenac
Enolic acids	Meloxicam, Piroxicam  Structure of Piroxicam
Fenamic acids	Meclofenamate, Mefenamic acid 

	Structure of Mefenamic acid
Napthylalkanones	Nabumetone  Structure of Nabumetone
Pyranocarboxylic acids	Etodalac  Structure of Etodalac
Pyrroles	Ketorolac

	 Structure of Ketorolac
COX-2 inhibitors	Celecoxib  Structure of Celecoxib

(USGyms.Net, 2010) (Fries James F., 1991)

Medicinal plants are used worldwide as an alternative and/or a complementary medicine. Studies on these medicinal plants including pharmacological and toxicological evaluations are essential for drug research and development. The main types of toxicological evaluations include: acute toxicity, subacute toxicity, subchronic toxicity, and chronic toxicity studies. Medicinal plants also can be poisonous, affecting the entire spectrum of organ systems, with some plants containing several toxic principles that affect different systems. Toxic principles can be found in different parts of medicinal plants: leaves, fruits, flowers, roots, and stem bark. In evaluating the acute toxicity or subacute toxicity of medicinal plants, any animal species can be used, though rodents are used most often.

1.4 Toxicity aspects of use of herbal preparations

Currently, there is an ongoing world-wide "green" revolution which is mainly premised on the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs

(Williamson *et al*., 1996). Many writers claim that it is assumed that "all things natural are good" (Gaillard and Pepin, 1999) and, generally, the extensive traditional use of herbal products is not assumed to be based on a comprehensive well documented logic, but rather on empirical wisdom accumulated over many years, often arrived at through trial and error and transmitted orally from generation to generation. This traditional methodology has enabled those herbal medicines producing acute and obvious signs of toxicity to be well recognized and their use avoided. However, the premise that "traditional use of a plant for perhaps many hundreds of years establishes its safety does not necessarily hold true". The more subtle and chronic forms of toxicity, such as carcinogenicity, mutagenicity, andhepatotoxicity, may well have been overlooked by previous generations and it is these types of toxicity that are of most concern when assessing the safety of herbal remedies (Williamson *et al*., 1996; Tomlinson and Akerele, 1998).

1.4.1 Causes 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).

1.4.2 Toxicology

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

1.4.3 Toxicity

Toxicity is the degree to which a substance can damage an organism. Toxicity can refer to the effect on a whole organism, such as an animal, bacterium, or plant, as well as the effect on a substructure of the organism, such as a cell (cytotoxicity) or an organ such as the liver (hepatotoxicity). By extension, the word may be metaphorically used to describe toxic effects on larger and more complex groups, such as the family unit or society at large.

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

1.4.3 Exposure

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

1.4.4 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)

1.4.5 Acute toxicity

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

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

1.4.6 Chronic toxicity

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

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

1.4.7 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).

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.

1.5.1 Cellular Elements of Blood

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

Blood is made of two parts:

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

1.5.2 Plasma

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

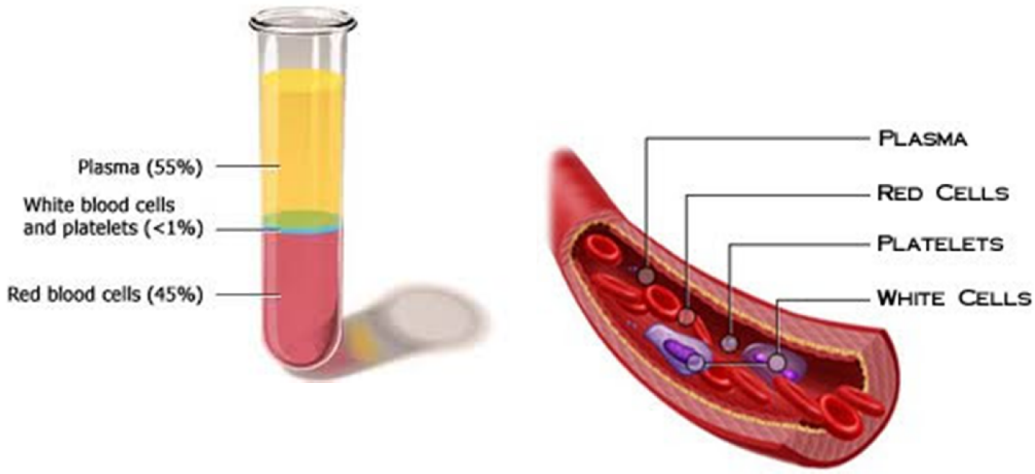
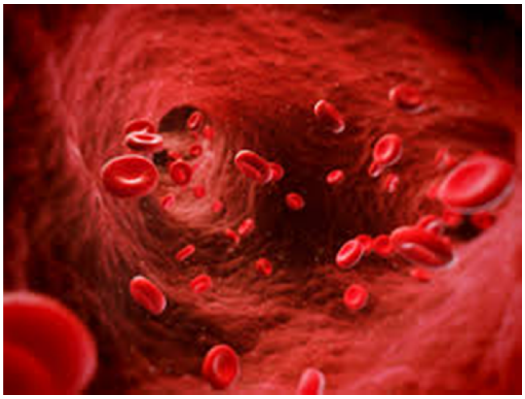


Figure-1.4: Plasma of the Blood (Alberts, 2012).

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).



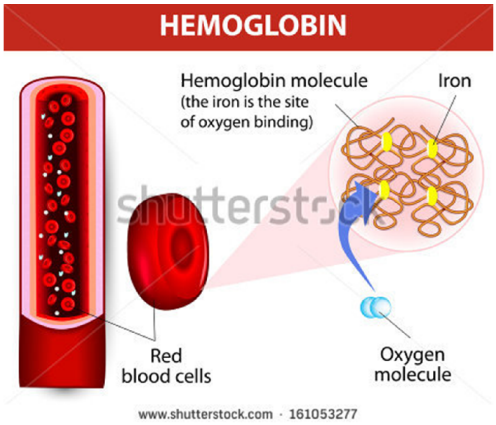


Figure-1.5: Red Blood Cell & Hemoglobin (Purves, 2004).

1.5.4.2 Different count of RBC

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

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

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

Normal results for adults vary, but in general are:

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

Lower than Normal Hemoglobin

Low hemoglobin level may be due to:

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

Higher than Normal Hemoglobin

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

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

Hematocrit (HCT)

The hematocrit (Ht or HCT, British English spelling haematocrit), also known as packed cell volume (PCV) or erythrocyte volume fraction (EVF), is the volume percentage (%) of red blood

cells in blood. It is normally 45% for men and 40% for women. It is considered an integral part of a person's complete blood count results, along with hemoglobin concentration, white blood cell count, and platelet count. Anemia refers to an abnormally low hematocrit, as opposed to polycythemia, which refers to an abnormally high hematocrit. Both are potentially life-threatening disorders (Purves, 2004).

Higher than Normal Hematocrit

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

Lower than Normal Hematocrit

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

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

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

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

Higher than Normal MCV

- In pernicious anemia (macrocytic), MCV can range up to 150 femtolitres.
- An elevated MCV is also associated 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).

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.

Mean corpuscular hemoglobin concentration (MCHC)

Mean corpuscular hemoglobin concentration (MCHC) is the average concentration of hemoglobin per unit volume of red blood cells and is calculated by dividing the hemoglobin by the hematocrit.

MCHC = $H_b / H_{ct} \times 100$

Normal range: 32-36 g/dL

When the MCHC is abnormally low they are called hypochromic, and when the MCHC is abnormally high, hyperchromic.

Red blood cell distribution width (**RDW** or **RCDW**)

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

Higher than Normal RDW

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

1.5.4.2 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 ente1r the tissues and become "mast cells" which help blood flow to injured tissues by the release of histamine.

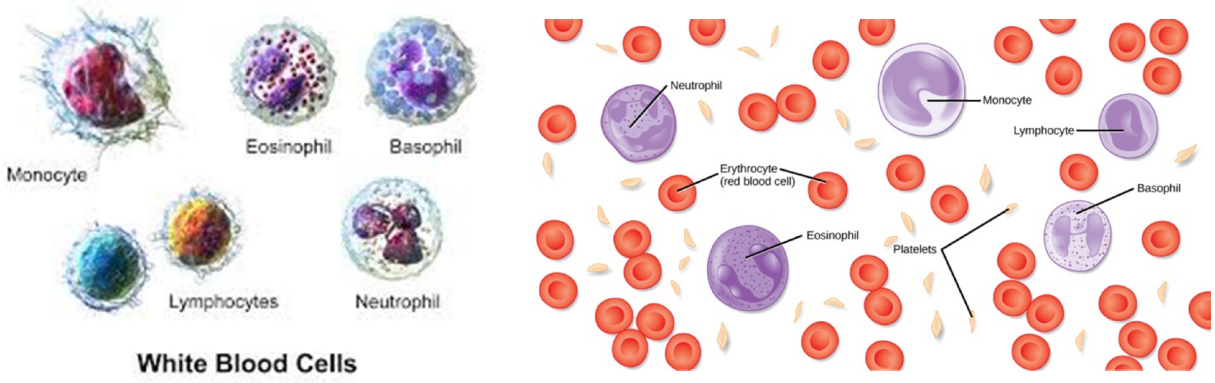


Figure1.6: Different Parts of White Blood Cell and Platelet (Ganong, 2003)

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).

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-3: Reference value of different protein that distinguish the liver disorders

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

(Ganong, 2003)

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 parenchyma cell. 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).

Chapter 2

Plant Introduction

2.1 Description of *Curcuma Caesia :*

Scientific name: *Curcuma Caesia*

Common Names :

Hindi: Kali Haldi,Krishna kedar;

Manipuri: Yaingang Amuba or Yaimu;

Marathi: Kala-haldi;

Telugu: Nalla Pasupu;

Kannada: kariarishina, naru kachora;

Bengali: Kala haldi;

Mizo: Aihang, Ailaihang;

Assamese: kala haladhi;

Nepalese Kaalo haledo;

Malayalam: Kari manjal;

Sanskrit: Rajani, Nishaa, Nishi, Raatri;

Malay: Black Haldi, Black curcuma, Kunyit Hitham, Temu Hitham;

Arabic: Gadwâr Aswad;

French: Zédoaire Noir;

German: Schwarze Zedoarwurzel;

Italian: Zedoaria Nera;

Turkish: Kara Cadvar

Taxonomic position

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida,

Order: Asterales

Family: Asteraceae

Genus: Mikania

Species: *Mikania cordata*

Curcuma caesia, black turmeric or black zedoary is a perennial herb with bluish-black rhizome, native to North-East and Central India. Black turmeric is also sparsely found in the Papi Hills of East Godavari, West Godavari of Andhra Pradesh and Khammam district of Telangana. The rhizome of black turmeric has a high economic importance owing to its putative medicinal properties. In west Bengal, the rhizome of the plant is used in Kali Puja, and hence the plant is called Kali haldi. By etymology, Kali is the feminine form of Kala, which means black color and hence the plant is termed as black turmeric in English. This species has been regarded as endangered by the central forest department of India due to biopiracy.

Chemical Constituents:

The research on the volatile rhizomes oil of Curcuma caesia resulted in the identification of 30 components,

1.Representing 97.48% of the oil,

2. camphor (28.3%),

3.ar-turmerone (12.3%),

4.(Z)ocimene (8.2%),

5. arcurcumene (6.8%),

6.1,8-cineole (5.3%),

6.elemene (4.8%),

7. borneol(4.4%),

8.bornyl acetate (3.3%)

9.curcumene (2.82%) as the major constituents.

2.1 Fig : Curcuma caesia

(Paliwal P, Pancholi S S, 2011)

2.2 Medical Uses :

The rhizomes are used as a rubefacient to rub the body after taking a Turkish bath. In Bengal, it is used in the fresh state-turmeric. The rhizomes of the herb are often used by the Baiga, Sahariya, Agariya, Gond, Korku, and other tribal communities of Mandla, Balaghat, Chhindwara, Anooppur, and Dindori district of Madhya Pradesh state for the treatment of pneumonia, cough, and cold in children, and for fever and asthma in adults. In northeast India, the powder of rhizomes is used by tribal women as a face-pack during their engagement and marriage period. Fresh rhizomes are crushed and applied as a paste on forehead for relief from migraine or applied on the body for sprains and bruises. In Lohit district of Arunachal Pradesh, Kanti tribes apply fresh rhizome paste on snake and scorpion bites .

Black Turmeric rhizome is believed to have magical powers. In Chhattisgarh, tribes make paste of rhizomes using cow's urine and apply the mixture on forehead as Bindi along with blood, for Vasikaran and Tantra practices. Some tribes believe that the rhizomes act as a talisman to keep evil spirits away. Some people believe that the rhizome of the plant is a form of the goddess Kali

and carry a rhizome in their pockets. Some Hindu organizations sell rhizome paste to use as Tilaka, with a belief that it will remove all kinds of black magic. The color of the root is considered similar to the skin complexion of several Hindu deities: Kali, Rama, Krishna, and Shiva.

2.3 Cultivation and harvest:

The cultivation and harvest practices are similar to that of common turmeric which is used in recipes. In the fields, the rhizomes are washed thoroughly and are placed in a wide mouthed cauldron. The water is poured in the cauldron such that the rhizomes are completely covered. The cauldron is covered with a lid, and the rhizomes are boiled for about 30 minutes until foam oozes out with a strong odour. The rhizomes are taken out when the water is reduced to one-third of the original and they are soft and their inner portion has turned from blue to dark or pale brown. The rhizomes are then dried in hot sun for10 to 15 days until hardened.

Macroscopy (Morphology of the Plant):The plant is usually erect ranging from 0.5 to 1.0 m in height; it is differentiated into underground large ovoid tuberous rhizome often called root-stock and an erect aerial shoot with leaves and flowers.

A.Rhizome: The rhizome is tuberous with camphoraceous sweet odor, about 2-6 cm in diameter, the shape and size is often variable. It is sessile, laterally flattened, and covered with adventitious roots, root scars, and warts; moreover, it shows longitudinal circular wrinkles on the surface giving the look of nodal and internodal zones to the rhizome. The surface (cork) of rhizome is dark brown, bluish black, or buff in color; it shows circular arrangements of remnants of scaly leaves, which gives a false impression of growth rings. The branching is more or less sympodral .

Root: As the plant propagates with rhizome, the primary roots are not noticed; however, yellow brown long fibrous and tapering adventitious roots are found all over the surface of rhizome.

Leaves: The leaves are in the groups of 10-20, each leaf is broad oblong lanceolate and glabrous. In the middle region the lamina shows deep farraginous purple colored clouds. The petiole is ivory color and ensheathing the petioles encircle each other forming a pseudoaxis. Inflorescence: It is 15-20 cm long dense spike, which arises much before the opening of leaf, the bracts are green, the bracts of coma are deep red, which become crimson when old . Flowers: Smaller than bracts, pale yellow with reddish border. Calyx: 10-15 mm long, obtuse. Root: The TS of the adventitious root is circular in outline. It shows

 Epiblema - Single layered. Consists of thick walled cutinized cells. In old specimen the epiblema is withered and is replaced by ten-layered rectangular cork cells

 Cortex - Heterogeneous differentiated into

a. Outer cortex - Composed of parenchymatous tissue of secondary and primary cortex
b. Middle cortex - Made up of radially arranged air chambers separated by one cell thick partition wall - the trabaculae (a character of hygrophilous plant)
c. Endodermis - In the innermost layer of the cortex, the cells are rectangular and barrel shaped.

 Pericycle - Three to four layered, consists of rectangular cells

 Vascular tissue - Radially arranged. Phloem patches and xylem are arranged alternately, xylem is exarch.

 Pith - Well developed and thick walled parenchymatous.

Rhizome: TS of rhizome triangular to circular,

 Epidermis - single layered composed of very thick wall cells, covered with thick cuticle

 Cortex - three to five layered, thick walled collenchymatous cells

 Endodermis - ill developed

 Pericycle - well-defined cells radially and compactly placed

 Pith - large parenchymatous, a large number of cells are filled either with starch grains or sphaeraphides, a number of vascular traces traverse in the pith may be leaf traces

 Vascular tissue - vascular bundles are conjoint and scattered, xylem consists of vessels and xylem parenchyma. Phloem composed of sieve tubes phloem parenchyma.

Leaf - the isobilateral leaf of plant shows:

 Epidermis - both upper and lower epidermis are identical, it is single and single layered covered with cuticle and perforated by stomatas

 Mesophyll - palisade and spongy parenchyma not demarcated, they are intermixed in mesophyll, and entire mesophyll is chlorophyllens with scattered oil cavities. The wall of oil cavities is well defined and made up of epithelial cells

 Vascular bundles - they are mixed with oil cavities, each bundle is conjoint and collateral with an arch of sclerenchyma over xylem.

Powder Study of Rhizome

The powder is brownish black with camphoraceous odor. The taste is bitter; it includes powder fibers and small granules of vessels .

 Parenchyma - spherical to angular cells in the forms of grains. The grains are clumps of parenchymatous cell; they are filled with starch grains, which become blue with iodine solution

 Oligorasin crystals - originally impregnated in parenchyma they become free in powder and are found in dispersed condition

 Vascular elements - large number of vessels elements either entire or in the form of fragments. They show spiral and pitted thickenings, most of the elements are of vessel category, and tracheids are few and occasional.

Chapter 3
Literature Review

3.1 Phytochemical studies :

3.1.1 Neuropharmacological activities:

Curcuma caesia Roxb. (Zingiberaceae), called black turmeric in English, is a perennial herb found throughout the Himalayan region, North-East and Central India. The plant has been traditionally used in India for several medicinal purposes. The present study was carried out to evaluate the methanol extract of *C. caesia* rhizome (MECC) for some neuropharmacological activities in experimental animal models.

3.1.2 Analgesic activity:

MECC (at 50 and 100 mg/kg body weight) was evaluated for analgesic activity by acetic acid-induced writhing and tail flick tests. Locomotor activity was measured by means of an actophotometer. Anticonvulsant property was assessed against pentylenetetrazol-induced convulsion in mice and muscle relaxant effect was evaluated by using rota-rod apparatus. The results of the present study revealed remarkable analgesic, locomotor depressant, anticonvulsant and muscle relaxant effects of *C. caesia* rhizome, demonstrating depressant action on the central nervous system. The outcome of present study can validate certain traditional uses of *C. caesia* rhizome in India.

3.1.3 Antioxidant Activity :

The methanol extract of *C. caesia* (MECC) rhizome for some *in vitro* antioxidant studies as because we know that many diseases are associated with reactive oxygen species (ROS) and reactive nitrogen species (RNS). Effect of MECC on ROS and RNS were evaluated in different *in vitro* methods like 1, 1-diphenyl-2-picrylhydrazil radical, hydroxyl radicals, superoxide anions, nitric oxide, hydrogen peroxide, peroxynitrite and hypochlorous acid. Lipid peroxidation, total phenolic content was also measured by standard assay method. The extract showed significant antioxidant activities in a dose dependent manner. The IC_{50} values for scavenging of free radicals were 94.03 ± 0.67 µg/ml, 155.59 ± 3.03 µg/ml, 68.10 ± 1.24 µg/ml, 21.07 ± 1.78 µg/ml, 260.56 ± 12.65 µg/ml and 33.33 ± 0.52 µg/ml for DPPH, nitric oxide,

superoxide, hydroxyl, peroxynitrite and hypochlorous acid respectively. Reductive ability of the extract was also tested where dose dependent reducing capability was observed. The rhizome extract contains 677.7 µg of phenolic compound in 10 mg of the extract which is accounted for its free radical as well as antioxidant activity. From the above study it is concluded that the methanol extract of *C. caesia* rhizome is a potential source of natural antioxidant. This study was undertaken to examine the antioxidant activity of methanolic extract of rhizomes of *Curcuma caesia* using DPPH free radical scavenging assay. The IC50 (The concentration of sample required to scavenge 50% of DPPH free radical) was calculated by plotting graph between % inhibition vs concentration. The Butylated Hydroxytoluene was used as standard antioxidant in comparison to methanolic extract of *Curcuma caesia*. The IC 50 value of extract and Butylated Hydroxytoluene was found to be 862.35 µgm and 46.25 µgm for 2 ml of 500 µM concentration of DPPH. This suggests that methanolic extract of *Curcuma caesia* had moderate IC 50 value as compared to Butylated Hydroxyltoluene.

3.1.4 Antifungal activity:

Rhizomes of *Curcuma caesia* are used medicinally in India. Essential oils extracted from the rhizomes were tested for antifungal activity against several human and plant pathogenic fungi. Dilutions of the oil in ethylene glycol were tested by an agar diffusion procedure on plates seeded with the test isolates. Some antifungal effect was noted, but no consideration was given in designing the experiment to the fact that oil diffused poorly through agar gels. The significance of the results therefore remains unclear. [A more logical choice would have been to incorporate the oil in gels at differing concentrations and measure growth from standard inocula.] *D. W. R. Mackenzie*.

3.1.5 Antimicrobial Activity :

The essential oil from rhizomes of *C. caesia*, rich in curcumene, ionone and turmerone, was tested for antimicrobial activity against *Bacillus subtilis*, *Corynebacterium diphtheriae*, *Staphylococcus aureus*, *Salmonella typhi*, *Escherichia coli*, *Shigella*, *Aspergillus niger*, *A. fumigatus*, *Curvularia lunata* [*Cochliobolus lunatus*], *Fusarium psidi* and *Rhizopus oryzae* by the paper disc diffusion method. The oil exhibited strong to moderate inhibitory action against all the

bacterial and fungal species tested. Activity was shown against all except *S. typhi* and *Shigella*, even at a 1:1000 dilution.

3.1.6 Antimutagenic Activity :

The rhizomes of Curcuma caesia Roxb. (zingiberacea) are traditionally used in treat-ment of various ailments and metabolic disorders like leukoderma, asthma, tumours,piles, bronchitis, etc. in Indian system of medicine. Considering the importance of natural products in modern phytomedicine, the antioxidant and antimutagenic activi-ties of C. caesia Roxb. rhizome extract and its fractions were evaluated. The ethanolic fraction showed highest antioxidant activity by DPPH assay (86.91%) comparable toascorbic acid (94.77%) with IC50 value of 418 _g/ml for EECC followed by MECC(441.90 _g/ml) > EAECC(561 _g/ml) > AECC(591 _g/ml). Based on the antioxidant activity, three of the rhizome extracts were evaluated for their antimutagenic properties against indirect acting mutagen cyclophosphamide (CP) using Salmonella typhimurium strains TA98and TA100. The antimutagenic activity of the extracts against indirect acting mutagen cyclophosphamide in the presence of mammalian metabolic activation system was found to be significant ($p < 0.01$, $p < 0.05$). All the extracts showed similar antimutagenicity in dose dependent manner. The total phenolic content as well as reducing ability of the extracts was also determined.

Chapter 4
Method and material

4.1 Preparation of plant extraction

The whole part of the plant was dried in room temperature for approximately two weeks. Then the dried plants were taken into fine powder by using a grinding machine. Then the extraction process was done. At first 5 kg dried plant dust of Curcuma Caesia was soaked in 26L methanol in 16 bottles. Then it was kept in room temperature for 7 days and everyday it was used to shake properly to ensure the maximum amount of constituents present in the grinded plant become soluble into methanol. After 7 days, the mixture was filtered. For filtration, white cotton cloth was used. After filtration two parts were obtained.

1.The residue portion over the filter.

2. The filtered part.

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

4.2 Crystal formation

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

4.3 Experimental Animals

Swiss mice of either sex (25-35 g) were obtained from the Animal house of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55-65%, r.t. 23.0±2.0°C and 12 h light: dark cycle). The animals were fed with standard diet and water adlibitum.

4.1 Fig: Swiss Albino Mice

4.4 CNS Activity Test

4.4.1 Materials for CNS Activity Test:

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

4.4.2 Chemical Agents Used in Test:

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

4.4.3 Doses Used in CNS Activity Test of the Extract:

1) Open Field Test:

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

4.4.4 Methods for CNS Activity Test:

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

- Open Field Test.

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.

3.4.5.1 Open Field Test:

3.2 Figure-: Open Field Test

1. 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).

2. The animals were divided into negative control and test groups containing ten mice in each group. Negative control group received vehicle (5% CMC solution) at a dose of 10 mg/kg body weight orally.

3. The test groups received extracts of *Curcuma Caesia* at the doses of 200,400 and 800 mg/kg body weight orally.

4. 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.

5. 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.

4.5 Toxicity Test

4.5.1 Materials for Toxicity Test

1. Analytical Balance,

2. Feeding needle: 1 c.c.

3. Insulin syringes 100 units disposable

4. 5 ml syringe disposable

5. Dissecting box

6. Dissecting pad

7. Pin

8. Beaker 1 litre

9. Petri dish for washing

10. Epindrop tube

11. 250 ml food grade plastic pot

12. Gloves

13 Mask

3.5.2 Chemical Agents Used in the Toxicity Test

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

2. Saline water (0.9%)

3. Formalin (5%)

4. EDTA

5. Heparin

4.5.3 Doses Used for Toxicological Activity of the Extract:

a) Acute Toxicity Test:

Methanolic extracts of *Curcuma Caesia* at a dose of 1000mg/kg, 1500mg/kg, 2000 mg/kg, 3000mg/kg and 6000mg/kg were administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses.

b) Chronic Toxicity Test:

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

4.5.4 Methods for Analgesic Activity Test:

a) Acute Toxicity Test

The acute toxicity of in Swiss albino mice was studied as reported method. Each extract were given to three groups (n = 10) of mice at 1000, 1500, 2000 and 3000, 6000 mg/kg body weight, orally. The treated animals were kept under observation for 3 days, for mortality and general behavior. (Paul,et.al. 2012).

b) Chronic Toxicity Test:

The adult Swiss albino mice were divided into four groups containing 10 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, 800 mg/kg according to body weight orally, respectively daily for 90consecutive 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 89th 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 10% formalin. Then this blood

and liver was used for the study of Hematology test, Protein Test and Liver biochemical parameters Test (Paul, et.al. 2012).

4.5.5 Hematological parameters

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

4.5.6 Serum biochemical parameters

Collected blood was used for the estimation of serum biochemical parameters like serum glutamate Oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline

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

4.5.7 Histopathological studies

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

4.5.8 Statistical Analysis

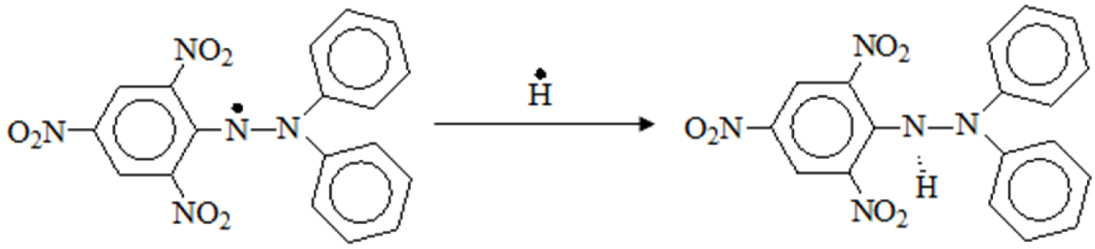
Data obtained from pharmacological experiments are expressed as mean±SEM. Difference between the control and the treatments in these experiments were tested for significance using one -way analysis of variance (ANOVA), followed by Dunnet's t-test for multiple comparisons using SPSS -16 software.

4.6 Determination of Antioxidant property

4.6.1 DPPH Free Radical Scavenging Assay

Principle

DPPH is a reactive free radical that acts as an electron acceptor (oxidant/ oxidizing agent) and causes oxidation other substances. On the other hand, antioxidants act as electron donors (reductant/ reducing agent). Antioxidants neutralize DPPH by being oxidized themselves. DPPH is found as dark-colored crystalline powder composed of stable free-radical molecules and forms deep violet color in solution. The scavenging of DPPH free radical (neutralization) is indicated by the deep violet color being turned into pale yellow or colorless.



1,.1-diphenyl-2-picrylhydrazyl **1,.1-diphenyl-2-picrylhydrazine**

Reagent	**Source**
Absolute Ethanol/Methanol	Merck, Germany
1,.1-diphenyl-2-picrylhydrazyl (DPPH	Sigma Chemicals, USA
Ascorbic acid (Analytical or Reagent grade)	SD Fine Chem. Ltd., Biosar, India

DPPH Solution: 0.004gm (4mg) DPPH is dissolved in 100 ml of solvent to make 0.004% solution.

4.6.1.1 : Preparation of Standard/ Extract solution

0.025 gm ascorbic acid or extract was taken and dissolved into 5 ml of Absolute ethanol. The concentration of the solution was 5mg/ml of ascorbic acid/ extact. The experimental concentrations from the stock solution were prepared by the following manner:

Concentration (µg/ml)	Solution taken from stock solution	Solution taken from others	Adjust the volume by Absolute ethanol	Final volume
800	320µl	-	1.68 ml	2.0 ml
400	-	1 ml(800µg/ml)	1 ml	2.0 ml
200	-	1 ml (400µg/ml)	1 ml	2.0 ml
100	-	1 ml (200µg/ml)	1 ml	2.0 ml
50	-	1 ml (100µg/ml)	1 ml	2.0 ml
25	-	1 ml (50µg/ml)	1 ml	1.0 ml
12.5	-	1 ml (25µg/ml)	1 ml	
6.25	-	1 ml (25µg/ml)	1 ml	

4.6.1.2 : Procedure

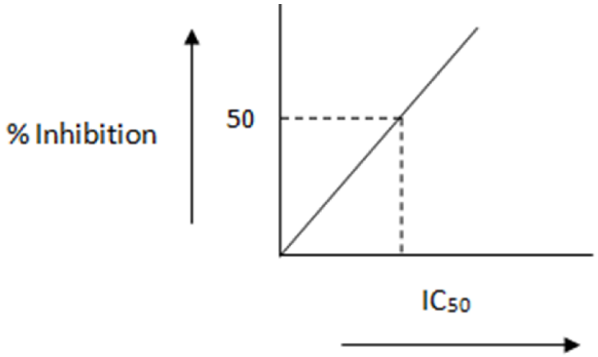
- The stock solution is serially diluted to achieve the concentrations of 400 µg/ml, 200 µg/ml, 100 µg/ml, 50 µg/ml, 25 µg/ml, 12.5 µg/ml
- Each test tube contains 1ml of each concentration and is properly marked

- 2 ml of 0.004% DPPH solution in the solvent is added to each test tube to make the final volume 3 ml (caution: DPPH is light sensitive, so making the solution and adding it to the test tubes should be done in minimum light exposure)
- Incubate the mixture in room temperature for 30 minutes in a dark place
- Then the absorbance is measured at 517 nm against dilute extract solution in the solvent

4.6.1.3 Calculation

$$\% \text{ Inhibition} = \left(1 - \frac{Absorbance\ of\ sample}{Absorbance\ of\ Control}\right) \times 100$$

IC_{50} is the concentration at which 50% of the total DPPH free radical is scavenged/ neutralized and can be determined by linear regression method from plotting % inhibition against corresponding concentration.



4.6.2 Determination of Total Phenolics Content

Principle

The content of total phenolic compounds of plant extracts was determined as described previously (Velioglu*et al.,* 1998) using the Folin-Ciocalteu Reagent (FCR). The Folin-Ciocalteu reagent (FCR) or Folin's phenol reagent or Folin-Denis reagent is a mixture of phosphomolybdate and phosphotungstate used for the colorimetric assay of phenolic and polyphenolic antioxidants

(Singleton*et al.,* 1999). It works by measuring the amount of the substance being tested needed to inhibit the oxidation of the reagent (Vinson *et al.,* 2005).

However, this reagent does not only measure total phenols and will react with any reducing substance. The reagent therefore measures the total reducing capacity of a sample, not just the level of phenolic compounds, Sequences of reversible one- or two-electron reduction reactions lead to blue species, possibly $(PMoW_{11}0_{40})^{4-}$. In essence, it is believed that the molybdenum is easier to be reduced in the complex and electron-transfer reaction occurs between reductants and Mo(VI):

Reagent	**Source**
Folin - ciocalteu reagent	Merck, Germany E.
Sodium carbonate	Merck (India) Limited
Methanol	Merck, Germany
Gallic acid	Sigma Chemicals, USA

4.6.2.1 Preparation of 7.5% Sodium carbonate solution

7.5 gm of Na_2CO_3 was taken into a 100 ml of a volumetric flask and the volume was adjusted by distilled water.

4.6.2.2 Preparation of Standard solution

The stock solution was prepared by taking 0.025 gm of galic acid and dissolved into 5 ml of Absolute Ethanol. The concentration of this solution was 5µg/µl of galic acid. The experimental concentrations from this stock solution were prepared by the following manner.

Concentration (µg/ml)	Solution taken from stock	Solution taken from others	Adjust the volume by distilled Ethanol (µl)	Final volume (ml)

	solution (µl)			
200	80	-	1920	2
100	-	1 ml (200 µl/ml)	1000	2
50	-	1 ml (100 µl/ml)	1000	2
25	-	1 ml (50 µl/ml)	1000	2
12.5	-	1 ml (25 µl/ml)	1000	2
6.25	-	1 ml (12.5 µl/ml)		2

4.6.2.3 Preparation of Extract solution

0.025 gm of each plant extracts were dissolved into 5 ml of Ethanol to make the concentration of each solution 5µg/µl of plant extract. These solutions were considered as stock solutions. The experimental concentration from these stock solutions was prepared by the following manner:

4.6.2.4 Experimental Procedure

1. 1.0 ml of plant extract (200µg/ml) or standard of different concentration solution was taken in a test tube.
2. 5 ml of Folin-Ciocalteu (Diluted 10 fold) reagent solution was added to the test tube.
3. 7.5% Sodium carbonate solution (4 ml) was added to the same test tube and mixed well.
4. Test tubes containing standard solutions were incubated for 30 minutes at 20°C to complete the reaction but the test tubes containing extract solution were incubated for 1 hour at 20°C to complete the reaction.
5. Then the absorbance of the solution was measured at 765 nm using a spectrophotometer against blank.
6. A typical blank solution contained the solvent used to dissolve the plant extract.
7. The Total content of phenolic compounds plant extracts in gallic acid equivalents (GAE)

was calculated using the following equation:

$$C = (c \times V)/m,$$

Where, C = total content of phenolic compounds, mg/gm plant extract, in GAE

c = the concentration of gallic acid established from the calibration curve (mg/ml)

V = the volume of extract in mlm = the weight of crude plant extract in gm

Chapter 5
Result and discussion

5.1 Consequences of dosing:

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

Fig 5 : Consequences of dosing

5.2 CNS Activity Test of Methanolic Extract of *Curcuma Caesia*

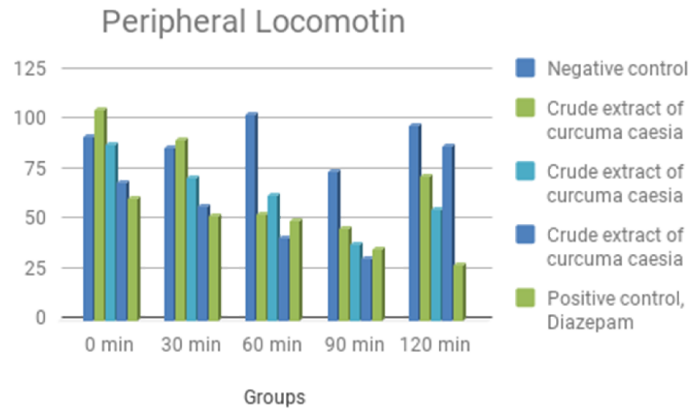
5.2.1 Open Field Test:

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

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

5.1: Table : CNS Activity of plant extract of *Curcuma Caesia* by Open Field Test (Peripheral Locomotion) in Mice.

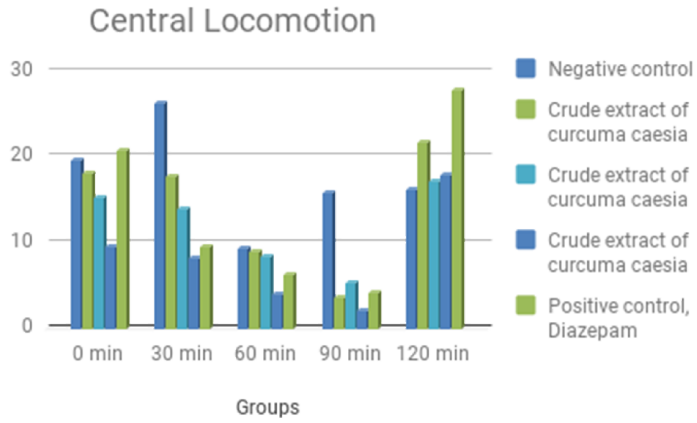
Groups	Dose	No. of Peripheral Locomotion				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	92.5±3.72	86.2±1.5	103.3±1.94	74.7±3.2	97.1±2.0
Crude extract of Curcuma Caesia	200mg/ Kg	105.0±1.4	89.9±1.9	53.0±2.84	46.1±1.5	71.7±2.1
Crude extract of Curcuma Caesia	400mg/ Kg	88.2±1.4	71.6±2.9	62.1±1.2	38.2±2.1	55.0±1.0
Crude extract of Curcuma Caesia	800mg/ Kg	68.5±4.2	57.5±2.7	40.9±3.0	31.0±1.9	86.8±1.7
Positive control, Diazepam	1mg/kg	60.83±1.1	52.33±1.1	50.0±1.8	35.67±1.1	27.83±1.7



5.1 Fig: Graphical Presentation of CNS Activity of Plant extract of *Curcuma Caesia* by Open Field test (Peripheral Locomotion) in mice.

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

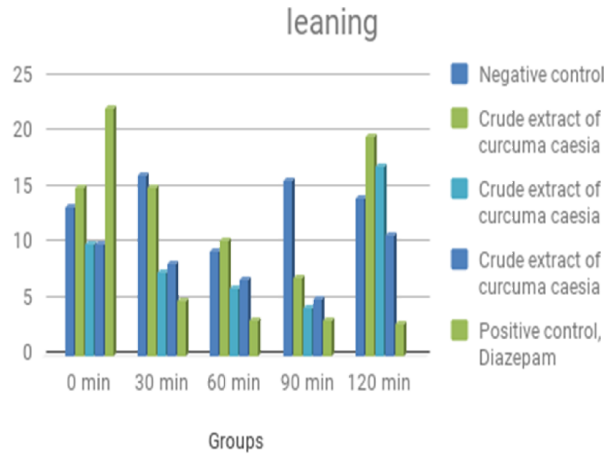
Groups	Dose	No. of Central Locomotion				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	19.5±.86	26.2±1.5	9.3±1.94	15.7±1.2	16.1±2.0
Crude extract of Curcuma Caesia	200mg/ kg	18.0±1.0	17.7±1.3	9.0±0.84	3.5±1.5	21.7±2.1
Crude extract of Curcuma Caesia	400mg/ kg	15.2±1.4	13.9±2.9	8.3±1.2	5.2±2.1	17.0±1.0
Crude extract of Curcuma Caesia	800mg/ kg	9.5±3.2	8.2±2.7	4.0±2.0	2.0±3.9	17.8±1.7
Positive control, Diazepam	1mg/kg	20.67±1.01	9.5±1.1	6.17±.76	4.17±1.1	3.33±.42



5.2: Figure : Graphical Presentation of CNS Activity of Plant extract of *Curcuma Caesia* by Open Field test.

5.3 Table: CNS Activity of plant extract of *Curcuam Caesia* by Open Field Test (Leaning) in Mice.

Groups	**Dose**	**No. of Peripheral Locomotion**				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	13.3±2.1	16.1±1.5	9.3±1.94	15.7±1.2	14.1±2.28
Crude extract of Curcuma Caesia	200mg/ kg	15.1±1.0	15.0±2.2	10.3±0.84	7.0±1.5	19.7±1.5
Crude extract of Curcuma Caesia	400mg/ kg	10.0±1.4	7.5±1.1	6.0±1.2	4.2±3.1	17.0±1.0
Crude extract of Curcuma Caesia	800mg/ kg	9.9±2.2	8.2±1.7	6.8±2.0	5.0±1.5	10.8±1.6
Positive control, Diazepam	1mg/kg	22.17±1.01	4.83±.31	3.17±.96	2.1±1.1	2.8±.4



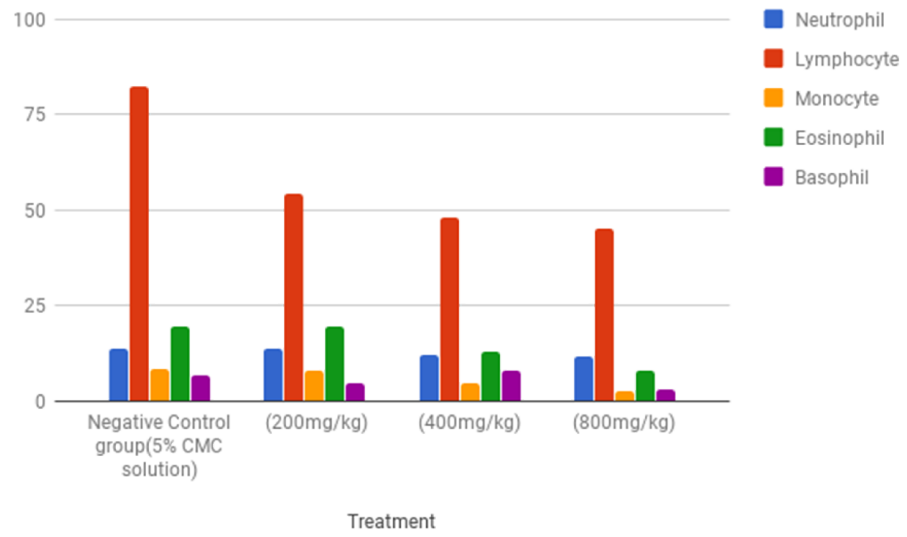
5.3 Figure: Graphical Presentation of CNS Activity of Plant extract of *Curcuma Caesia* by Open Field test.

CBC (Count Blood Cell) Test, Biochemical Test:

Drug dose was 200,400 and 800 mg/kg (CBC &Biochemical Test).In the chronic study of methanolic extract of *Curcuma Caesia* at a dose (200,400,800 mg/kg) to the mice, significant difference were not found in the erythrocyte and leucocytes values of both the treated and control mice. In which case, the administration of *Curcuma Caesia* methanolic extract for a period of 90 days cannot induce significant aneamia. Though minor irregularities were observed mainly in the RBC, WBC, Neutrophil, Platelet, SGPT, SGOT and (hepatic enzymatic test) this could be as a result of the mice response to foreign bodies associated with the chronic toxicity during the experiment.

5.4 Table : Effect of *Curcuma Caesia* on the different count of WBC (White Blood Cell)

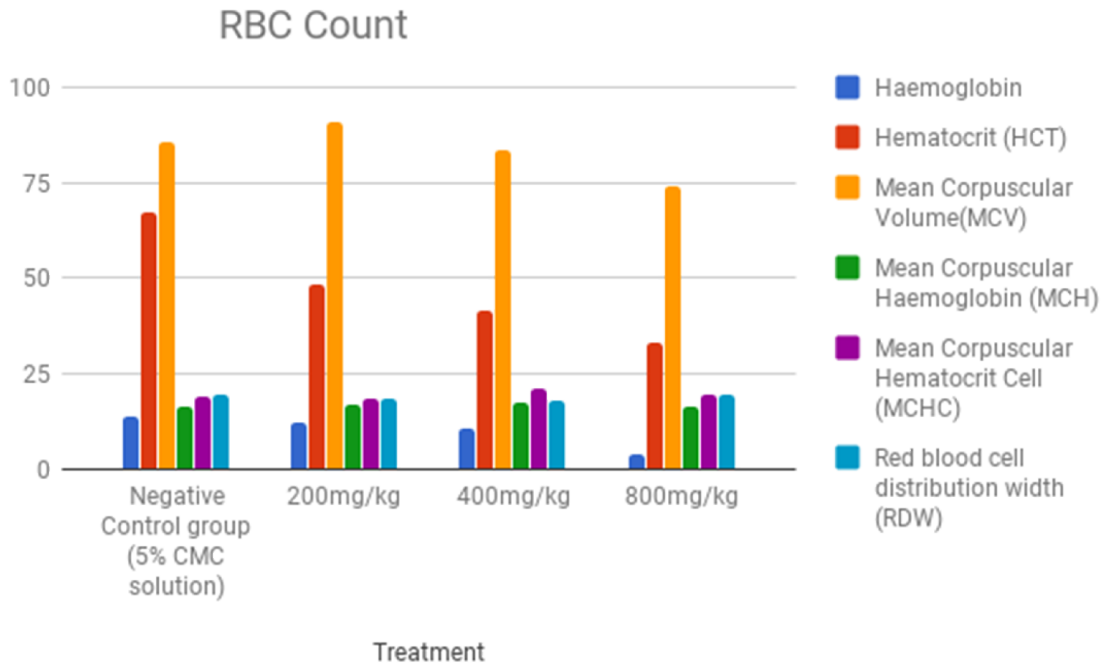
Treatment Group	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Negative Control group(5% CMC solution)	13.9	82.2	8.25	19.65	6.96
***Curcuma Caesia* (200mg/kg)**	13.6	54.2	7.9	19.45	4.8
***Curcuma Caesia* (400mg/kg)**	12	48.15	4.7	12.96	8.23
***Curcuma Caesia* (800mg/kg)**	11.65	45	2.85	8	3.05



5.4 Fig : Graphical representation *Curcuma Caesia* on the different count of WBC

5.5 Table: Effect of *Curcuma Caesia* on the Different count of RBC (Red Blood Cell)

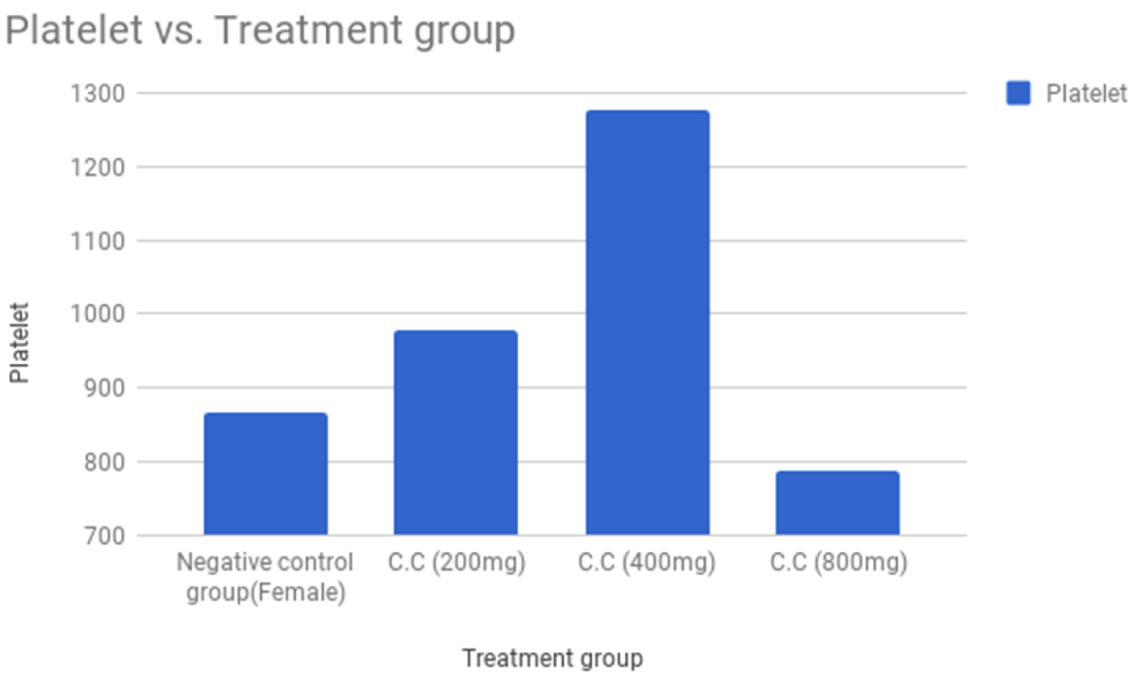
Treatment Group	Haemoglobin	Hematocrit (HCT)	Mean Corpuscular Volume(MCV)	Mean Corpuscular Haemoglobin (MCH)	Mean Corpuscular Hematocrit Cell (MCHC)	Red blood cell distribution width (RDW)
Negative Control group(5% CMC solution)	13.9	67.17	85.8	16.35	19	19.37
Curcuma Caesia **200mg/kg**	12.2	48.55	90.65	16.85	18.65	18.45
400mg/kg	10.7	41.73	83.63	17.45	21.15	18.26
800mg/kg	3.77	33.05	73.8	16.55	19.86	19.86



5.5 Fig : Graphical representation *Curcuma Caesia* on the different count of RBC

5.6 Table : Effect of *Curcuma caesia* on Platelet count on the CBC

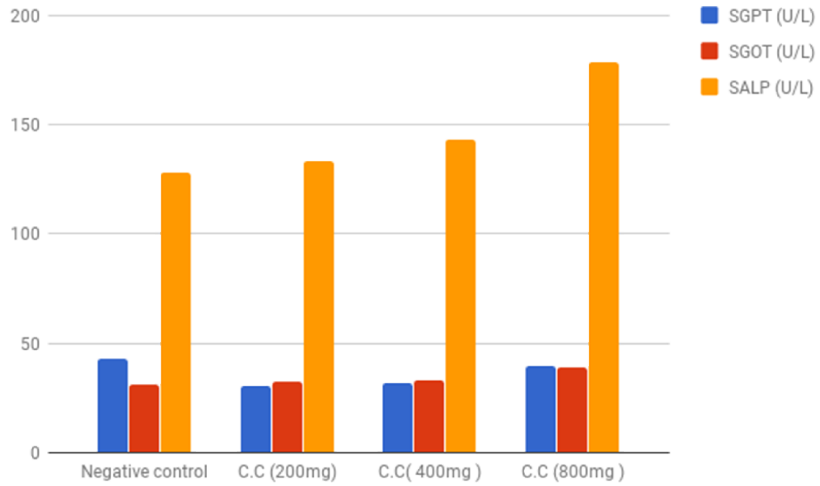
Treatment group	Platelet
Negative control group(Female)	867.4
***Curcuma Caesia* (200mg)**	979.4
***Curcuma Caesia* (400mg)**	1277.38
***Curcuma Caesia* (800mg)**	789



5.6 Figure : Graphical Presentation of CNS Activity of Plant extract of on Platelet.

5.7 Table : Effect of *Curcuma Caesia* on the Liver Function Test

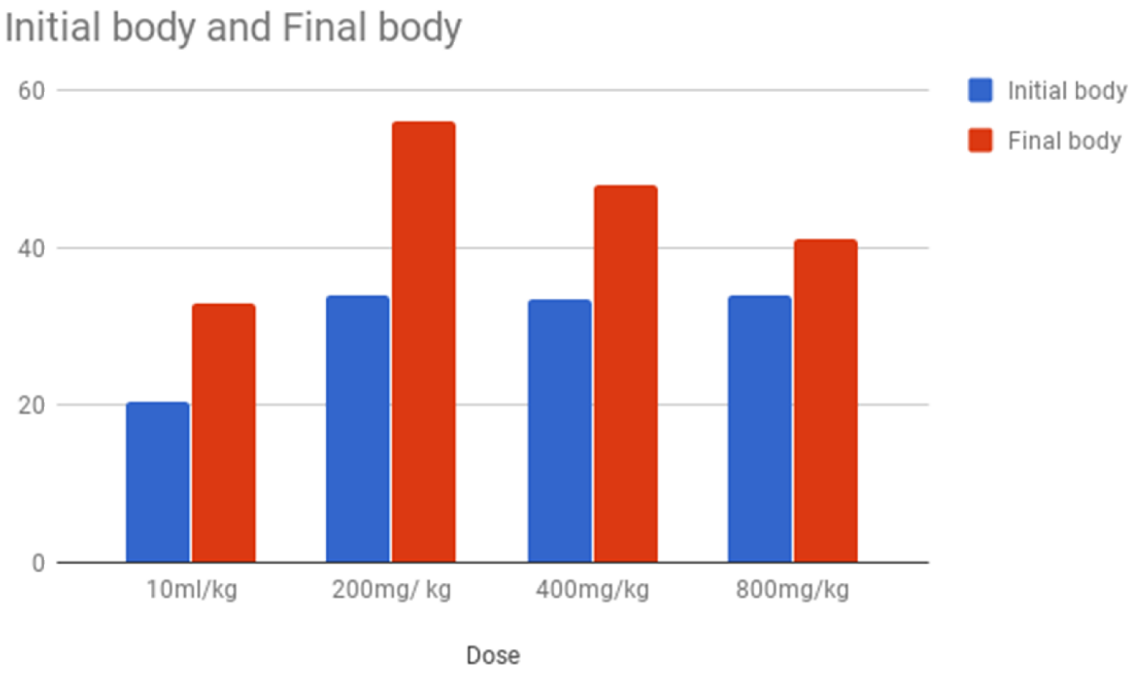
Treatment group	Dose	SGPT (U/L)	SGOT (U/L)	SALP (U/L)
Negative control	group(Male)	10ml/kg 42.6	30.8	128
***Curcuma Caesia* (200mg)**	200mg/kg	30.4	32.7	133.5
***Curcuma Caesia*(400mg)**	400mg/kg	31.9	33.2	142.95
***Curcuma Caesia* (800mg)**	800mg/kg	39.9	38.7	178.4



5.7 Figure : Graphical Presentation of CNS Activity of Plant extract of *Curcuma Caesia* on liver function test.

5.8 Table : Effect of *Curcuma Caesia* on Body weight in mice

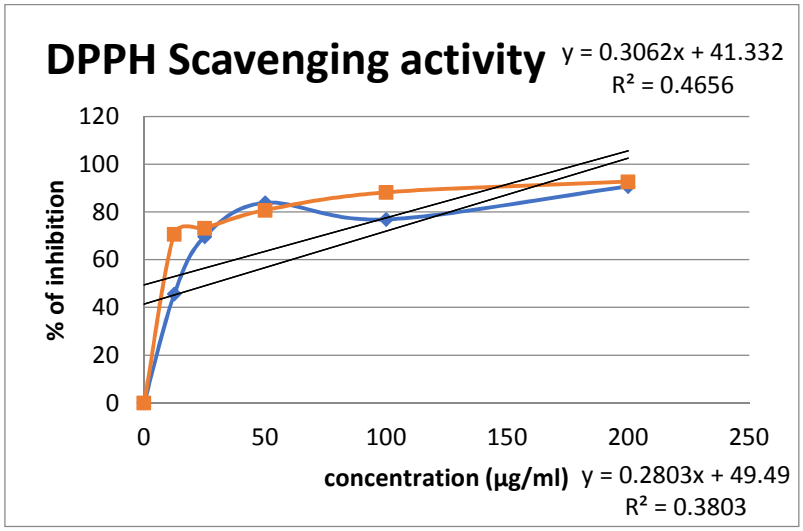
Treatment Group	Dose	Initial body	Final body
Negative control group(Female)	10ml/kg	20.5±0.6	32.8±1.93
Curcuma Caesia (200mg)	200mg/ kg	34.0±0.95	56±2.6
Curcuma Caesia (400mg/kg)	400mg/kg	33.3±1.9	48±1.2
Curcuma Caesia (400mg/kg)	800mg/kg	33.9±0.86	41±2.0



5.8 Figure : Graphical Presentation of Effect of methanolic extract of *Curcuma Caesia* on bodyweight in mice.

Result of DPPH Scavenging activity test :

Leaf methanol extract of *Curcuma caesia* showed significant amount of DPPH scavenging activity compared with ascorbic acid .The percent of inhibition of standard was monitored highest at the concentration of 200 µg/ml which was 92.7105.The percent of inhibition of leaf methanol was also highest at the concentration of 200 µg/ml which was 90.69767.The result shows that the leaf extract of methanol has high anti-oxidant or free radical inhibition property .



5.9 Fig : Graphical Representation of DPPH Scavenging Activity test where red line is for sample and blue line is for standard

Table 5.9 : DPPH Scavenging activity for Methanolic extract of *Curcuma Caesia :*

Concentration (µg/ml)	Leaf methanol Absorbance	% of inhibition of Leaf methanol
0	0	0
12.5	0.468	45.5814
25	0. 261	69.65116
50	0.139	83.83721
100	0.08	76.86047
200	0.058	90.69767

Table 5.10 : DPPH Scavenging activity of Ascorbic acid :

Concentration (µg/ml)	Standard Absorbance	% of inhibition of Standard
0	0	0
12.5	0.399	70.66349
25	0.352	73.25201
50	0.233	80.74978
100	0.766	88.18804
200	0.24	92.7105

5.11 Table :Determination of IC_{50} :

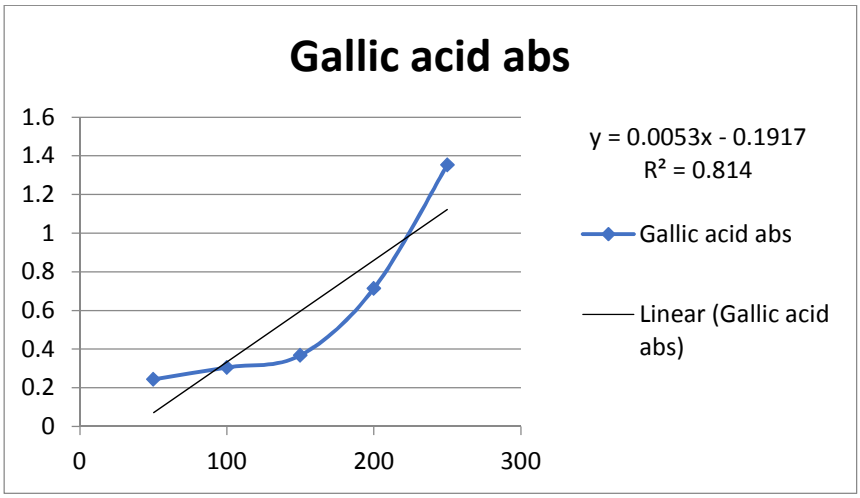
Methanol extract *C. caesia* /Ascorbic acid	Regression Line	R^2 Line	IC_{50} Value (µg/ml)
Methanol extract of *C. caesia*	Y=0.3062x+41.332	R^2=0.4656	28.30
Ascorbic acid	Y=0.2803x+49.49	R^2=0.3803	1.8194

Total Phenolic content Test :

Phenolic compound of plant acts as primary antioxidants or free radical scavenger . *Curcuma caesia* extract in methanol showed high concentration of total phenolic content.

Table 5.12: Absorbance Data of Total phenolic content test :

Test Tube No.	**Leaf methanol**
1.	0.996
2.	0.995
3.	0.783



5.10 Fig: Total Phenolic content 223.1333±24.5375 (mg/g) gallic acid equivalent

Table 5.6 : Determination of Mean and Standard deviation :

Methanolic extract of *C.caesia* Absorbance	**Y=0.005x-0.191**	**Mean**	**Standard Deviation**	**Total phenolic content (mg/g)**
0.996	237.4			
0.995	237.2	223.1333	24.53759	223.1333±24.5375
0.783	194.8			

Discussion :

Traditional medicines are mostly utilized by means of the natural products isolated from natural resources such as plant extracts. Pharmacological studies always reveal the potential medicinal properties of plants of our surroundings. Ethnobotanical data on the traditional uses of plants encourage the isolation of secondary metabolites leading to new lead compounds. With the increasing demands of inventing new drugs the pharmacological assay of natural plant resources play an unparallel role in traditional drug discovery. Day by day the study of traditional medicinal plants is increasing in significant rate with the view to invention and establishment of new therapy line. The plant extract was also assessed on the central nervous system using a number of neuro-pharmacological experimental models in mice. The crude extract of *Curcuma caesia* (200mg/kg, 400mg/kg & 800mg/kg) dose dependently reduces the number of peripheral locomotion, central locomotion and leaning in the open field test. The reduction is significant (***$p<0.001$) when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Diazepam 1mg/kg. The crude extract of *Curcuma caesia* (200mg/kg, 400mg/kg& 800mg/kg) also dose dependently reduces the number of head dipping and head poking in the hole board test. The reduction is significant (***$p<0.001$) when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Diazepam 1mg/kg. The reference drug is found slightly potent than the extract. The aim of the study was also to investigate the possible toxicity of the plant *Curcuma caesia* and especially to establish the safety of the methanolic extract of this plant by focusing on its chronic toxicity in mice. For finding chronic toxicity several tests are done such as CBC (Cell Blood count) test, Hepatic enzyme test and histopathological Studies. CBC test and hepatic enzyme test are done by hematological machine and histopathological studies by microscopic test. The results of several widely accepted protocols would suggest that there were positive modulations in all the parameters of study in the *Curcuma caesia* extract, in which significant difference were not found in RBC and different count of RBC. In which case, the administration of *Curcuma caesia* methanolic extract for a period of 90 days cannot induce significant aneamia. .All results were found normal in the WBC, Neutrophil, lymphocyte, Platelet, SGPT, SGOT and ALP (hepatic enzymatic test) test. And the view of all organs were normal (Histopathology tests are not yet done). But the body weight and appetite of the mice of 200 mg was increased highly and then 400 mg and 800 mg respectively. From the present investigation, it can be concluded that the

methanolic extract of *Curcuma caesia* exhibited Depressant activity, and do not show any toxicity for male mice. But it may has metabolic disorder in low dose (200mg) and that's why body weight was increased.

Methanol extract of leaf of *Curcuma caesia* showed significant level of activity in DPPH scavenging test. The IC_{50} values were obtained by linear regression analysis of the dose response curves, which were plots of % inhibition versus concentration. The IC50 value for the sample was 28.30 whereas IC_{50} the standard is 1.8194. IC_{50} is the concentration of an inhibitor where the response (or binding) is reduced by half. In this test we have found that the IC_{50} value of sample is much higher than the ascorbic acid which shows high DPPH scavenging activity.

Free radicals, generated in the human body as metabolic by-products or acquired from the environment, have been claimed to play a key role in affecting human health by causing oxidative damages associated with many degenerative diseases such as coronary heart diseases, atherosclerosis, aging, cancer and inflammatory conditions .In phenolic test the results showed that generally, the methanol extracts of leaf contained high levels of total phenolic contents. So this test also confirms about the antioxidant property.

Conclusion :

Curcuma caesia Roxb. is a perennial, erect rhizomatous herb with large leaves. Fresh rhizomes are aromatic with intense camphoraceous odour, cultivated for its rhizomes, which are used in traditional medicine. The plant is reported to contain camphor, ar-turmerone, (Z)-ocimene, ar-curcumene, 1, 8-cineole, elemene, borneol, bornyl acetate and curcumene as the major constituents. The plant has been reported to have antioxidant activity, analgesic, locomotor depressant. It is now considered as a valuable source of unique natural products for development of medicines against various diseases. This review gives a view mainly on the medicinal uses and pharmaceutical uses.

References:

Amalraj VA, Velayudhan KC, Muralidharan VK (1989) A note on the anomalous flowering behaviour in *Curcuma caesia* (Zingiberaceae). J Bombay Nat History Soc 86:278–279

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

Aziz .S . and Feeroz. C. (2010). Utilization of forest flora by Phayre'sleaf-Monkey Trachypithecusphayrei (Primates: Cercopithecidae) in semi-evergreen forests of Bangladesh. *Journal of Threatened Taxa, 1(5),* pp.257-262.

Baghel, S., Baghel, R., Sharma, K. and Sikarwar, I., (2013). Pharmacological activities of Curcuma caesia. *International Journal of Green Pharmacy*, *7*(1), p.1

Behar, N., Tiwari, K.L. and Jadhav, S.K., (2014). A Review on Non-Conventional Turmeric: Curcuma caesia Roxb. *Current Trends in Biotechnology & Pharmacy*, *8*(1).

Beniddir .eBorgne . orga B. oa c, N., Lozach, O., Meijer, L., Awang, K. and Litaudon, M., (2014). Acridone Alkaloids from Glycosmischlorosperma as DYRK1A Inhibitors. *International Journal of Natural Products, 77(5),* pp.1117-1122.

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

Carrubba, 2012 ,Carrubba, A.; Scalenghe, R. (2012). "Scent of are Nostrum — Medicinal and Aromatic Plants (MAPs) in Mediterranean soils". *Journal of the Science of Food and Agriculture. 92 (6)*: 1150–1170.

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

Cheek, M. (2004). "Garcinia kola".IUCN Red List of Threatened Species.Version 2008.*International Union for Conservation of Nature.*Accessed on 21 may 2017.

Collins, Minta (2012) Medieval Herbals: The Illustrative Traditions. *University of Toronto Press.*p. 32.

Cravotto G.; Boffa L.; Genzini L.; Garella D. (2010). "Phytotherapeutics: an evaluation of the potential of 1000 plants". *J Clin Pharm Ther. 35 (1):* 11–48.

Das, S., Bordoloi, P.K., Phukan, D. and Singh, S., (2012). Study of the anti-ulcerogenic activity of the ethanolic extracts of rhizome of Curcuma caesia (eecc) against gastic ulcers in experimental animals. *Asian J Pharm Clin Res*, *5*(2), pp.200-203.

Devi, H.P., Mazumder, P.B. and Devi, L.P., 2015. Antioxidant and antimutagenic activity of Curcuma caesia Roxb. rhizome extracts. *Toxicology Reports*, *2*, pp.423-428.

Devi, H.P. and Mazumder, P.B., (2016). Methanolic extract of Curcuma caesia Roxb. Prevents the toxicity caused by cyclophosphamide to bone marrow cells, liver and kidney of mice. *Pharmacognosy research*, *8*(1), p.43.

Dewangan, M.K., Dwivedi, C., Lal, P., Singh, S. and Sinha, D., 2014. Medicinal value of curcuma cassia Roxb: an overview. *Earth journals*, *3*(4), pp.1-9.

Dr.ChiranjitParmer,(2008),Frutipedia; *Encyclopedia of edible Fruits around the world,* online [Available at http://www.fruitipedia.com/index.]. Accessed on 21 may 2017.

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

Garg, S.C. and Jain, R.K., 1998. Antimicrobial efficacy of essential oil from Curcuma caesia. *Indian Journal of Microbiology*, *38*(3), pp.169-170.

Hossan, S., Agarwala, B., Sarwar, S., Karim, M., Jahan, R. and Rahmatullah, M., (2010).Traditional use of medicinal plants in Bangladesh to treat urinary tract infections and sexually transmitted diseases.*Ethnobotany Research and Applications, 8,* pp.061-074.

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

Israr, F., Hassan, F., Naqvi, B.S., Azhar, I., Jabeen, S. and Hasan, S.M.F., 2012. Studies on antibacterial activity of some traditional medicinal plants used in folk medicine. *Pakistan journal of pharmaceutical sciences*, *25*(3).

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

Jena, B.S., Jayaprakasha, G.K. and Sakariah, K.K., (2002). Organic acids from leaves, fruits, and rinds of Curcuma caesia.*Journal of Agricultural and Food chemistry, 50(12),* pp.3431-3434.

Joseph, G.S., Jayaprakasha, G.K., Selvi, A.T., Jena, B.S. and Sakariah, K.K., (2005). Antiaflatoxigenic and antioxidant activities of Garcinia extracts. *International Journal of Food Microbiology, 101(2),* pp.153-160.

Kala, Prakash. C.,Sajwan (2007). "Revitalizing Indian systems of herbal medicine by the National Medicinal Plants Board through institutional networking and capacity building".*Current Science. 93 (6):* 797–806.

Karmakar, I., Saha, P., Sarkar, N., Bhattacharya, S. and Haldar, P.K., (2011). Neuropharmacological assessment of Curcuma caesia rhizome in experimental animal models. *Oriental Pharmacy and Experimental Medicine*, *11*(4), pp.251-255.

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

Krishnaraj, M., Manibhushanrao, K. and Mathivanan, N.,(2012). A comparative study of phenol content and antioxidant activity between non-conventional Curcuma caesia Roxb. and Curcuma amada Roxb. *International Journal of Plant Production*, *4*(3), pp.169-174.

Lichterman, B. L. , (2004) , "Aspirin: The Story of a Wonder Drug". *British Medical Journal. 329 (7479): 1408*

Likhitwitayawuid, K., Phadungcharoen, T. and Krungkrai, J., (1998).Antimalarial xanthones from Curcuma caesia. *Planta medica, 64(01),* pp. 70-72.

Likhitwitayawuid, K., Phadungcharoen, T., Mahidol, C. and Ruchirawat, S., (1997). 7-OMethylgarcinone E from Curcuma caesia.*Phytochemistry, 45(6),* pp.1299-1301.

Liu, Y., Qiu, Y.P., Zhang, L. and Chen, J., (2005). Dormancy breaking and storage behavior of Curcuma caesiaRoxb.(Guttiferae) seeds: implications for ecological function and germplasm conservation. *Journal of Integrative Plant Biology, 47(1),* pp.38-49.

Liu, Y., Chen, J., Bai, Z., Deng, X. and Zhang, L., 2001.Seed dispersal, seed predation, and seedling spatial pattern of Curcuma caesia (Guttiferae).*ActaPhytoecologicalSinica, 26(4),* pp.427-434.

Mayoclinic.org. (2016). Elevated liver enzymes - Mayo Clinic. [online] Available at: http://www.mayoclinic.org/symptoms/elevated-liver-enzymes/basics/definition/sym-20050830 [Accessed 27 oct. 2017].

Merck Manuals Consumer Version.(2016). Lymphocytic Leukocytosis. [online] Available at: http://www.merckmanuals.com/home/blood-disorders/white-blood-cell-disorders/lymphocyticleukocytosis [Accessed 27 oct. 2017].

Mohit, M., Jainender, J. and Minakshi, K., 2010. In-vitro evaluation of antioxidant activity of Curcuma caesia Roxb. *International Journal of Pharmaceutical Sciences and Research (IJPSR)*, *1*(9 Suppl.),pp.98-102.

Murugesan, K., Chemical Composition and Antioxidant Activity of Essential Oil from Curcuma caesia, *International Journal of Pharmaceutical Biology,* pp. 15

Muthukrishnan, J. and Ananthagowri, B., (1994). Botanical Pesticides and Energetics in The Control of The Castor Semilooper Achaeajanata Linnaeus (Noctuidae: Lepidoptera). Phytophaga (Madras), . *International Journal of Pharmaceutical Biology,* 6(2), pp.127-131.

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

Negi, P.S., Jayaprakasha, G.K. and Jena, B.S., (2008).Antibacterial activity of the extracts from the fruit rinds of Curcuma caesia and Garciniapedunculata against food borne pathogens and spoilage bacteria.*LWT-Food Science and Technology, 41(10),* pp.1857-1861.

Negi, P.S., Jayaprakasha, G.K. and Jena, B.S., (2010).Evaluation of antioxidant and antimutagenic activities of the extracts from the fruit rinds of Curcuma caesia.*International Journal of Food Properties, 13(6),* pp.1256-1265.

Nekvindová, J ,2007, Nekvindová, J.; Anzenbacher, P. (July 2007). "Interactions of food and dietary supplements with drug metabolising cytochrome P450 enzymes".*CeskaSlov Farm. 56 (4):* 165–73.

Panthong, K., Pongcharoen, W., Phongpaichit, S. and Taylor, W.C., (2006).Tetraoxygenatedxanthones from the fruits of Curcuma caesia.*Phytochemistry, 67(10),* pp.999-1004.

Paul, N. Roy, R. Bhattacharya, S. Biswas, M., (2012).Acute and Sub-chronic Toxicity Study of Cocosnucifera Leaf Extracts in Mice, *Journal of Advanced Pharmacy Education & Research*, pp. 74-81.

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

Pillai, D.S., Prabhasankar, P., Jena, B.S. and Anandharamakrishnan, C., (2012). Microencapsulation of Curcuma caesia fruit extract and effect of its use on pasta process and quality. *International Journal of Food Properties, 15(3),* pp.590-604.

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

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

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

Rahmatullah, M., Mollik, A.H., Ahmed, N., Bhuiyan, Z.A., Hossain, M., Azam, N.K., Seraj, S., Chowdhury, M.H., Jamal, F., Ahsan, S. and Jahan, R., (2010). A survey of medicinal plants used by folk medicinal practitioners in two villages of Tangail district, Bangladesh.*AmericanEurasian Journal of Sustainable Agriculture,* pp.357-363.

Randeep, G., Vandna, K. and Amandeep, S., 2011. Phytochemical investigation and evaluation of anthelmintic activity of Curcuma amada and Curcuma caesia-A comparative study. *J Ethnopharmacol*, *2*, pp.1-4.

Sahu, R. and Saxena, J.,(2013). A brief review on medicinal value of Curcuma caecia. *International journal of pharmacy & life sciences*, *4*(5).

Sarangthem, K. and Haokip, M.J., 2010. Bioactive components in Curcuma caesia Roxb. grown in Manipur.

Sahu, R. and Saxena, J., 2013. Screening of total phenolic and flavonoid content in conventional and non-conventional species of curcuma. *Journal of Pharmacognosy and Phytochemistry*, *2*(1).Sawant, S.B., Bihani, G., Mohod, S. and Bodhankar, S., 2014. Evaluation of analgesic and anti-inflammatory activity of

methanolic extract of curcuma caesia roxb. rhizomes in laboratory animals. *Int J Pharm Pharm Sci*, *6*(2), pp.243-247.

Shen, J. and Yang, J.S., (2006). Two new xanthones from the stems of Curcuma caesia.*Chemical and pharmaceutical bulletin, 54(1),* pp.126-128.

Shen, J., Tian, Z. and Yang, J.S., (2010). The constituents from the stems of Curcuma caesiaRoxb.and their cytotoxic activities. *Die Pharmazie-An International Journal of Pharmaceutical Sciences, 62(7),*pp.549-551.

Siridechakorn, I., Phakhodee, W., Ritthiwigrom, T., Promgool, T., Deachathai, S., Cheenpracha, S., Prawat, U. and Laphookhieo, S., (2012). Antibacterial dihydrobenzopyran and xanthone derivatives from Curcuma caesia stem barks. *Fitoterapia, 83(8),* pp.1430-1434.

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

Sumner, Judith ,(2000), The Natural History of Medicinal Plants. *Timber Press.* p. 17

Wahyuni, F.S., Byrne, L.T., Dianita, R., Jubahar, J., Lajis, N.H. and Sargent, M.V., (2004).A new ring-reduced tetraprenyltoluquinone and a prenylatedxanthone from Curcuma caesia.*Australian journal of chemistry, 57(3),* pp.223-226.

WONG, L.P. & KLEMMER, P.J. (2008): Severe lactic acidosis associated with juice of the mangosteen fruit, Garciniamangostana. *American Journal of Kidney Diseases 51(5):* 829833.

Xu, G., Kan, W.L., Zhou, Y., Song, J.Z., Han, Q.B., Qiao, C.F., Cho, C.H., Rudd, J.A., Lin, G. and Xu, H.X., (2010). Cytotoxic acylphloroglucinol derivatives from the twigs of Curcuma caesia.*Journal of natural products, 73(2),* pp.104-108.