Pharmacological and Toxicological Studies of Methanolic Extract of *Mikania cordata* and *Spilanthes acmella*

This Thesis Paper Submitted in Partial Fulfillment of the Requirement for the Degree of Masters of Pharmacy, East West University

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This Research paper is dedicated to my beloved Mother

DECLARATION BY THE CANDIDATE

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

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ENDORSEMENT BY HEAD OF THE DEPARTMENT

This is to certify that the dissertation entitled "**Pharmacological and Toxicological studies of Methanolic Extract of *Mikania cordata* and *Spilanthes acmella***" is a genuine research work carried out by Farjana Haque, under the supervision of Shamsun Nahar Khan (Ph. D, Postdoc, Harvard University, Chairperson, Department of Pharmacy, East West University, Dhaka). I further certify that no part of the thesis has been submitted for any other degree and all the resources of the information in thus connection are duly acknowledged.

Dr. Shamsun Nahar Khan
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CERTIFICATE

This is to certify that, the thesis on "**Pharmacological and Toxicological studies of Methanolic Extract of *Mikania cordata* and *Spilanthes acmella***" submitted to Department of Pharmacy, East West University, Aftabnagar, Dhaka, in partial fulfillment of the requirements for the degree of Masters of Pharmacy (M. Pharm), was carried out by Farjana Haque (ID # 2012-3-79-022) under my guidance and supervision and that no part of the thesis has been submitted for any other degree. We further certify that all the sources of information of in this connection are duly acknowledged.

_____

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List of Contents

Chapter 2

Chapter 3

List of Figures

List of Tables

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Statement of Purpose

The research was carried out in order to characterize the pharmacological & toxicological profile of the methanolic extract of ***Mikania cordata*** and ***Spilanthes acmella.***

The test was done on the following aspect:

Central Nervous System Activity:

- Open Field Test
- Hole Board Test

Analgesic Activity:

- Acetic Acid Induced Writhing Test.
- Formalin Induced Paw licking Test.

Toxicity Profile Estimation:

- Acute Toxicity Test
- Sub-chronic Toxicity Test
    - ✓ Haematological Studies
    - ✓ Biochemical Estimation
    - ✓ Histopathological Studies

Abstract

The plants which are useful for healing several diseases are called medicinal plant. Use of these plants for therapeutic purposes has been in practice in this country since time immemorial. Herbal toxicity is a field that has rapidly grown over the last few years along with increased use of herbal products worldwide. The plant, *Mikania cordata* (Bum.f) B.L. Robinson, is well known medicinal plant amongst traditional practitioner in Bangladesh, for its medicinal values that treat several local illness. The plant extract was assessed on the central nervous system using a number of neuropharmacological experimental models in mice. The methalonic extract of *Mikania cordata* at 200 mg/kg and 400mg/kg showed significant decreased activity in the open field test and hole board test which confirms the depressant activity. The methanolic extract of *Mikania cordata* of 800mg/kg, 1200 mg/kg, n-hexane, DCM fraction has been used to assess the analgesic and anti-inflammatory activity properties using Acetic acid induced writhing test and Formalin-induced paw licking test. *Mikania cordata* can significantly attenuate acetic acid-induced writhing episodes and acute and delayed phases of formalin-induced pain in mice in

dose dependent manner comparable to that produced by Indomethacin and aspirin respectively. The methanolic extract of *Spilanthes acmella* of n-hexane, chloroform, ethyl acetate, water fraction has been used to assess the analgesic and anti-inflammatory activity properties using Acetic acid induced writhing test and Formalin-induced paw licking test. Chloroform fraction significantly attenuate acetic acid-induced writhing episodes and acute and delayed phases of formalin-induced pain in mice. In the present study, the safety profile of *Mikania cordata* was evaluated by acute and sub-chronic toxicity study in Swiss albino mice. In acute toxicity study, each extract up to 6000 mg/kg body weight orally did not produce any toxic effect or death. In sub-chronic toxicity study, the three dose of 200mg/kg, 400mg/kg, 600mg/kg were administered for 45th day. The hematological, histological, serum and hepatic biochemical parameters were evaluated by sacrificing the animals. Mortality was observed during the course of study period. Number of decreased cell count observed in hematological, number of SGPT increased in biochemical, and number of cell also decreased in histological parameters in treated groups when compared to vehicle control group after 45 days. The results of the present study therefore indicated that *Mikania cordata* has potent depressant activity, potent analgesic activity but not safe in adult *Swiss albino* mice demonstrating noticeable toxicity.

Key Words: Medicinal plant, Open Field, Hole Board, Analgesic, Anti-inflammatory, *Mikania cordata,* Writhing, Licking, Acute Toxicity, Sub-chronic Toxicity.

Chapter 1

Introduction

Chapter 2

Introduction of Plant

Chapter 3

Material & Method

Chapter 4

Results and Discussion

Chapter 5

Conclusion

Reference

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 (Varalakshmi, et.al. 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 (Hall, et.al. 2012).

1.1.1 Definitions of medicinal plants

A considerable number of definitions have been proposed for medicinal plants. According to the WHO, "A medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis." When a plant is designated as 'medicinal', it is implied that the 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, 2003).

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).

1.1.3 Medicinal plants & Traditional Medicine Practice in Bangladesh

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

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

Traditional medical practice among the tribal people is mainly based on the use of plant and animal parts and their various products as items of medicine. The medicaments, prepared from plant materials and other natural products sometimes also include some objectionable substances of animal origin. They are dispensed in a number of dosage forms like infusions, decoctions,

pastes, moulded lumps, powders, dried pills, creams and poultices. Diets are strictly regulated (Hussain, et.al. 2012).

1.1.4. Nervous System

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

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

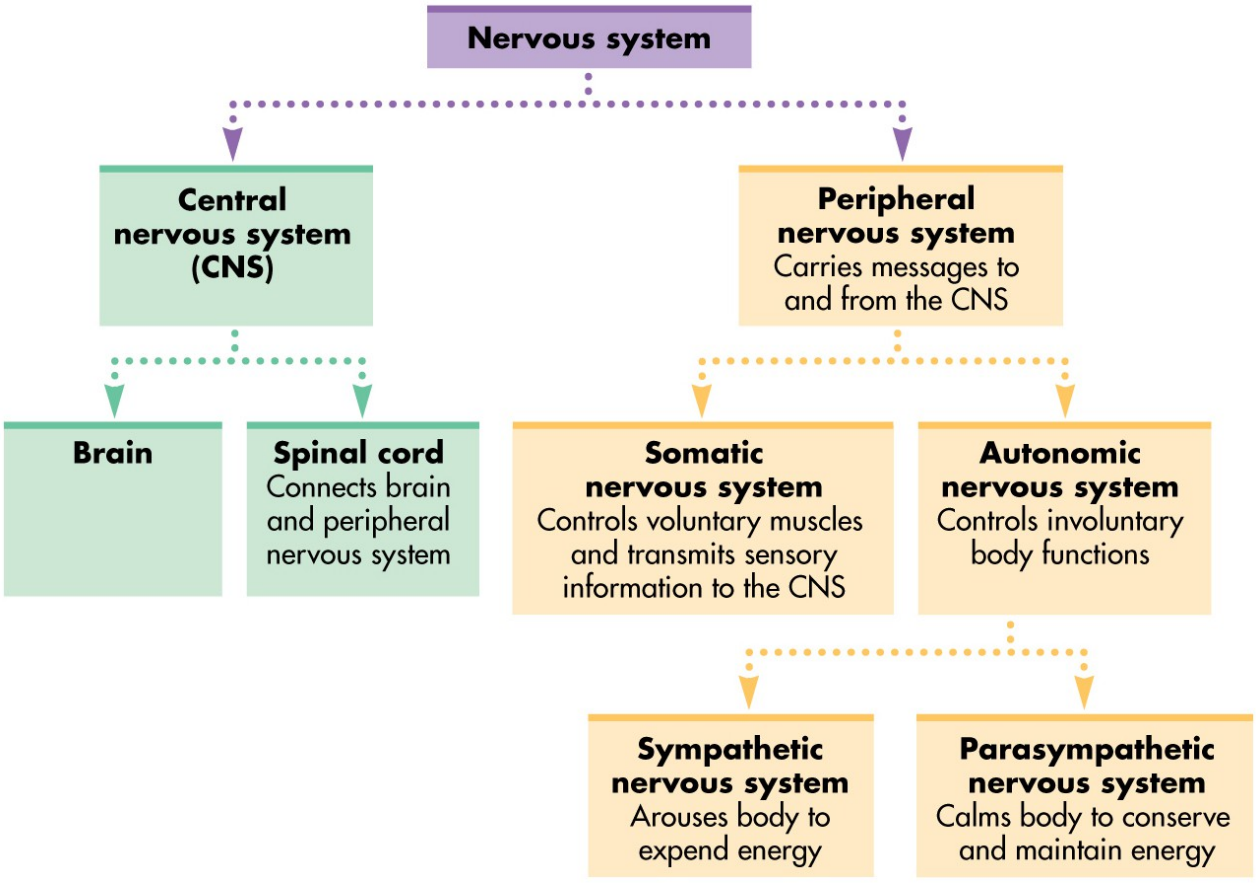


Figure-1: Organization of the Human Nervous System.

1.1.4.1. The central Nervous System

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

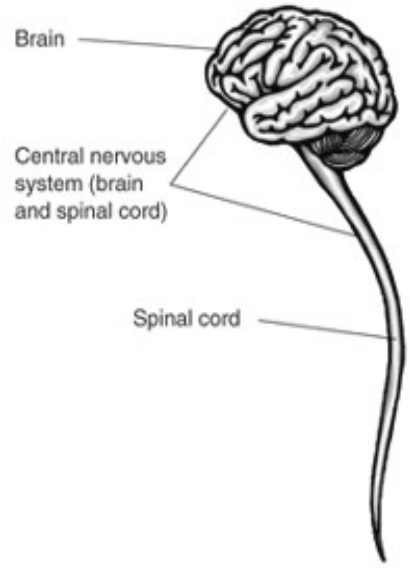


Figure-2: Central Nervous System

1.1.4.2. Parts of Central Nervous System

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

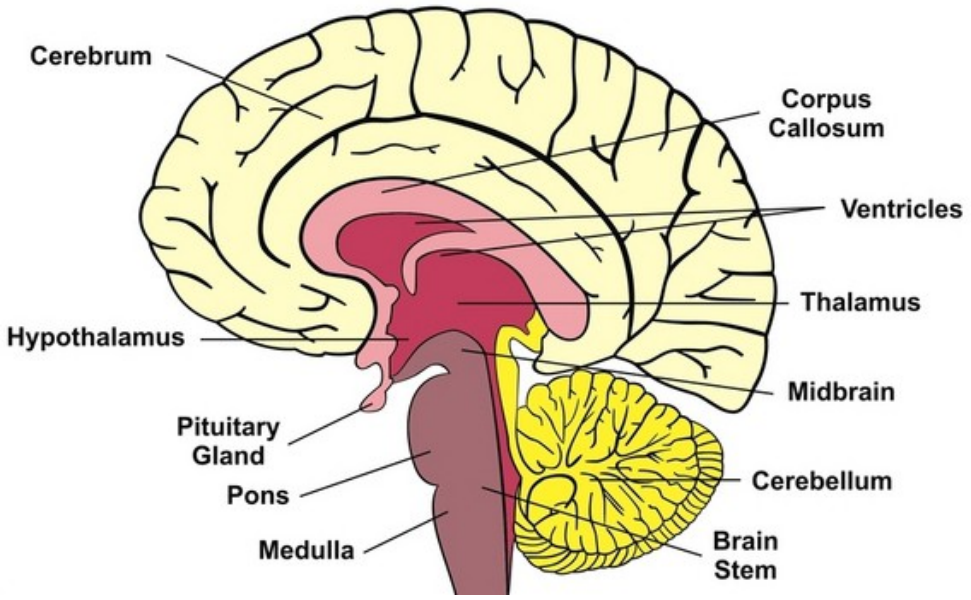


Figure-3: Human Brain

1.1.4.3. Peripheral Nervous System:

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

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

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

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

1.1.4.4. Nerve cells

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

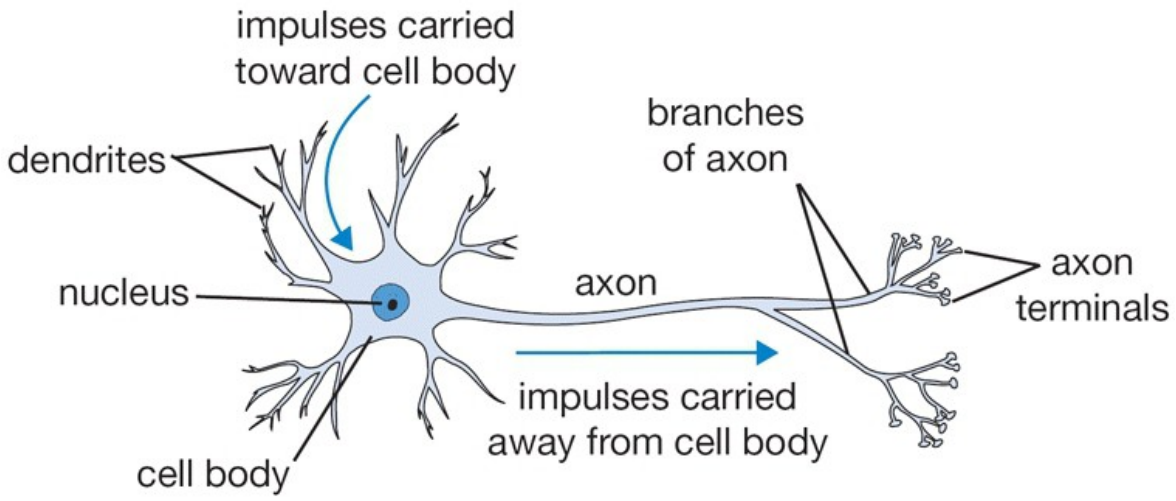


Figure-4: Neuron

Cell Body or Soma:

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

Axon:

Cell body gives rise to a tubular process which is the main conducting unit of the neuron, capable of conveying information at great distances by propagating transient electrical signal called action potential. Many axons are surrounded by a segmented, white, fatty substance called myelin or the myelin sheath. Myelinated fibers make up the white matter in the CNS, while

cell \bodies and unmyelinated fibers make the gray matter. The unmyelinated regions between the myelin segments are called the nodes of ranvier. Thus, axons are of two types, myelinated and non-myelinated.

Dendrites:

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

1.1.4.5. Synapse

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

1.1.4.6. Different Central Nervous System Disorders

- ✓ **Alzheimer's disease**-A progressive, degenerative disease that occurs in the brain and results in impaired memory, thinking, and behavior.
- ✓ **Bradykinesia**- Slowness of movement.
- ✓ **Bradyphrenia**-Slowness of thought processes
- ✓ **Cerebral embolism**- A brain attack that occurs when a wandering clots (embolus) or some other particle forms in a blood vessel away from the brain - usually in the heart.
- ✓ **Cerebral hemorrhage**- A type of stroke occurs when a defective artery in the brain bursts, flooding the surrounding tissue with blood.
- ✓ **Cerebral thrombosis**- The most common type of brain attack; occurs when a blood clot (thrombus) forms and blocks blood flow in an artery bringing blood to part of the brain.
- ✓ **Delusions**- A condition in which the patient has lost touch with reality and experiences hallucinations and misperceptions.

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

1.2 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, et.al. 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.

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 (Halliwell, and Gurtteridge, 1990).

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 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 and function 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

It 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 (WHO, 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.

.

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.

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.

1.2.3.2 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.

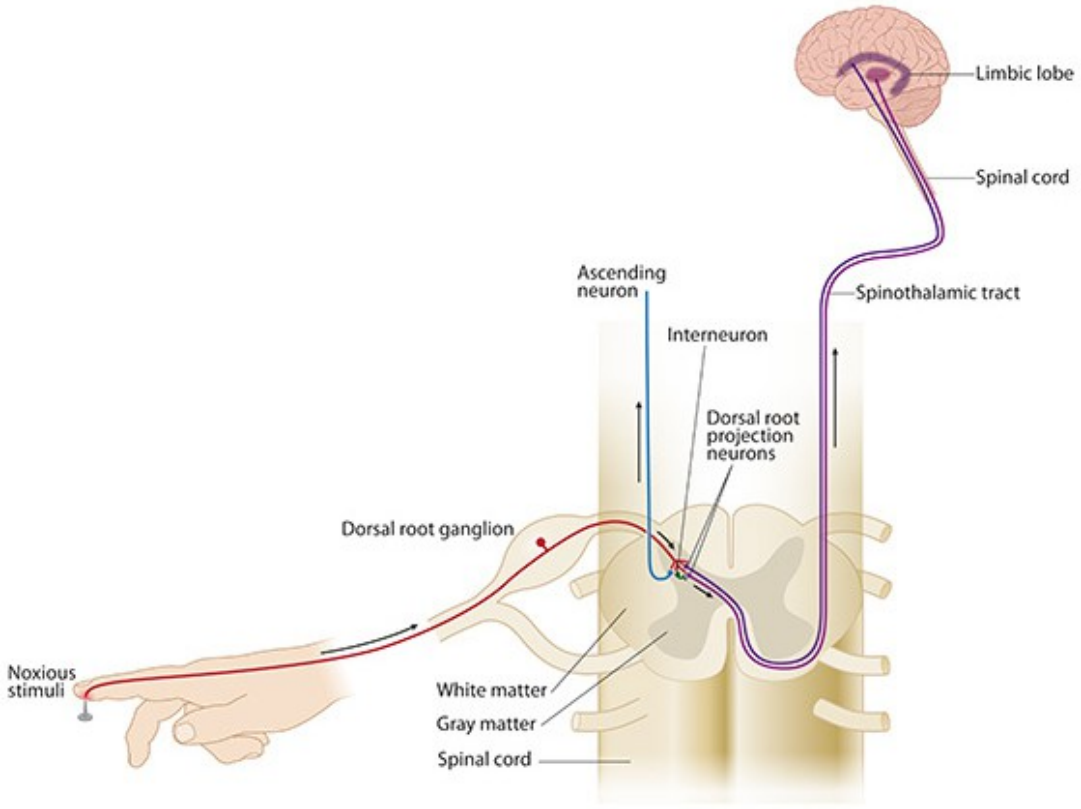


Figure-5: Transmission of Pain

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.

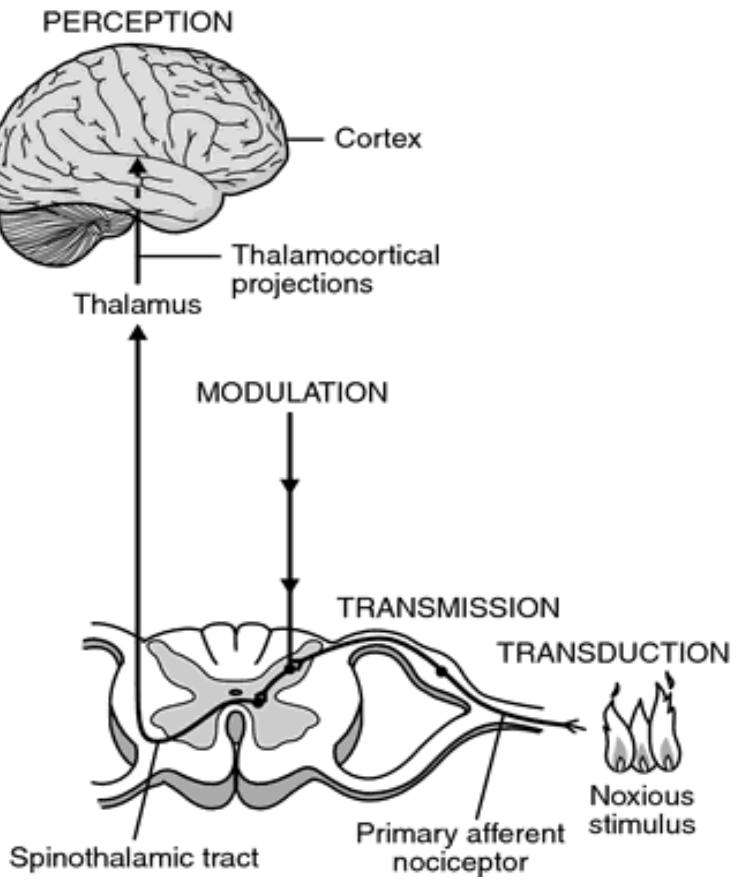


Figure-6: Pain Pathway and Mechanism.

1.2.3.3 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).

1.2.3.4 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, et.al. 2004).

1.2.5 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 pain message rapidly; if the pain is relatively mild, the pain 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, et.al. 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

- **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, et.al. 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.

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 (Piper, et.al. 1991).

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 & phase II mechanisms & others by 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, 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

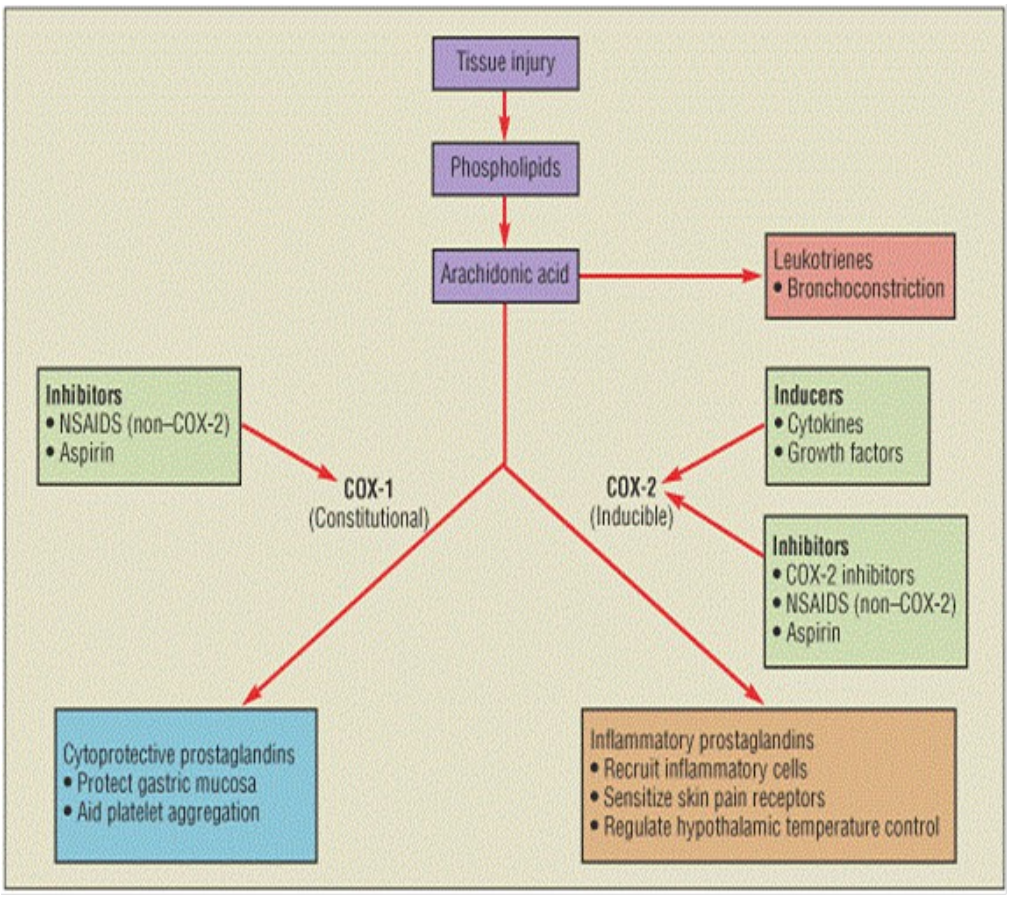


Figure-7: Pathway of inhibition of NSAIDs (Steven, 2003).

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, 1996).

1.3.4 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.

1.3.5 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**.**

1.3.6 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 (Solomon, et.al. 2010).

1.3.7 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 (Solomon, et.al. 2010).

NSAIDs cannot be used in the following cases:

- When patient has an allergy to aspirin or any NSAID
- During pregnancy
- During breast feeding

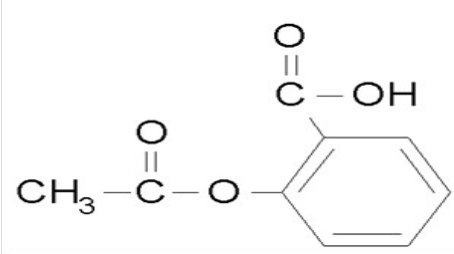
- While patient is on blood thinning agents (anticoagulants)
- Suffering from a defect of the blood clotting system (coagulation)
- While patient has an active peptic ulcer
- While patient is being treated for a fracture use should be monitored.

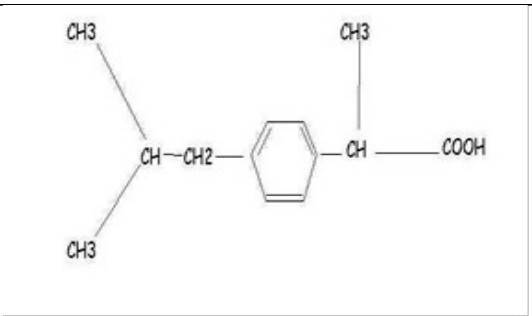
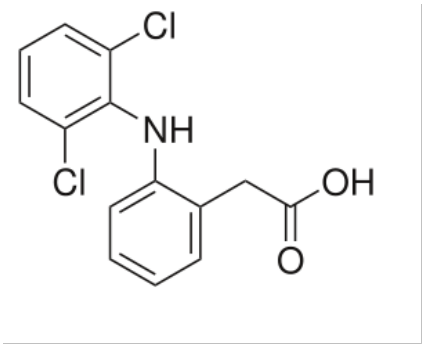
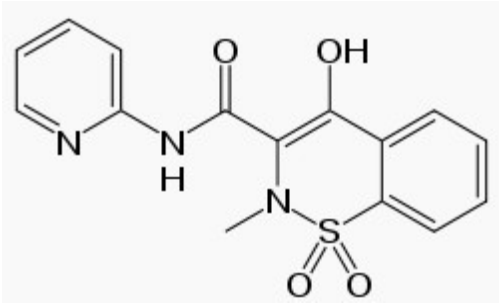
Care is needed if one has:

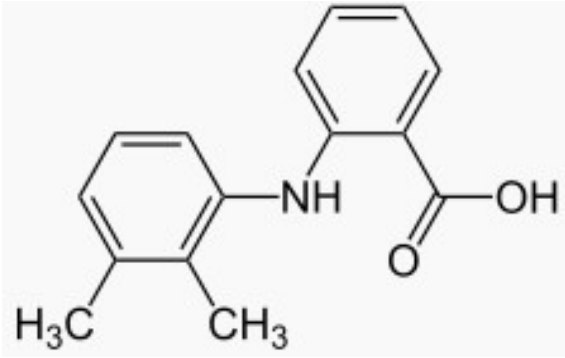
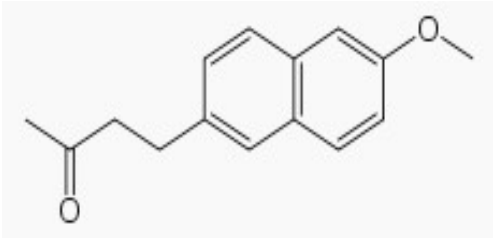
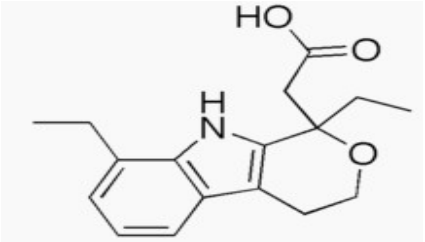
- Asthma
- Liver impairment
- Heart impairment
- Kidney impairment (James ,1991)

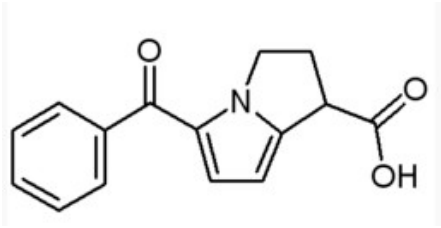
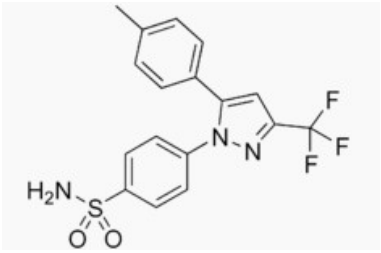
1.3.8 Classification of NSAIDs

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

(James, 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, sub-acute toxicity, sub-chronic 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 sub-acute 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, and hepatotoxicity, may well have been overlooked by previous generations and it is these types of toxicity that are of most concern when assessing the safety of herbal remedies (Williamson *et al*., 1996).

1.4.1 Causes of toxicity with herbal products

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

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

• In the first category, as previously mentioned, the toxicity may be as a result of exposure to intrinsic ingredients of some medicinal plants. Examples of some more important classes of ingredients implicated here include: *pyrrolizidine alkaloids,* which are said to be hepatocarcinogens; *aristolochic acid I*, said to be mutagenic and carcinogenic; *phorbol esters,*

which are tumor promoters and vesicant to the skin; *carboxy actractyloside*, a deadly toxic compound; *amygdalin*, a cyanogenic compound with many undesired effects; etc. (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 bacterial and 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 establish the extent of damage in living organisms. The relationship between dose and its effects on the exposed organism is of high significance in toxicology.

1.4.3 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.

1.4.4 Exposure

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

1.4.5 Route of Exposure

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

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

1.4.6 Acute toxicity

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

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

1.4.7 Chronic toxicity

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

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

1.4.8 Evaluation of herbal toxicity

Herbal toxicity can be evaluated by

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

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

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

1.5 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 (Ramsay, 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, 1998). 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 in the mid 1950's for blood cell counting. The research was based on the technique known as "Coulter's Principle" or the Aperture Impedance technique. This technique uses the resistivity of the blood cells because thee impedance of the cells suspended in the diluting fluid is much more higher than that of fluid was based on the fact that the resistivity of blood cells is much higher than that of the diluting fluid. Most modern cell counters serves on the basis of this extensively developed since 1950's.

1.5.2 Cellular Elements of Blood

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

Blood is made of two parts:

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

1.5.3 Plasma

Plasma is made up of 90% water, 7-8% soluble proteins (albumin maintains bloods osmotic integrity, others clot, etc), 1% carbon-dioxide, and 1% elements in transit. One percent of the plasma is salt, which helps with the pH of the blood. The largest group of solutes in plasma contains three important proteins to be discussed. There are: albumins, globulins, and clotting

proteins. Plasma also carries Respiratory gases; CO2 in large amounts (about 97%) and O2 in small amounts (about 3%), various nutrients (glucose, fats), wastes of metabolic exchange (urea, ammonia), hormones, and vitamins.

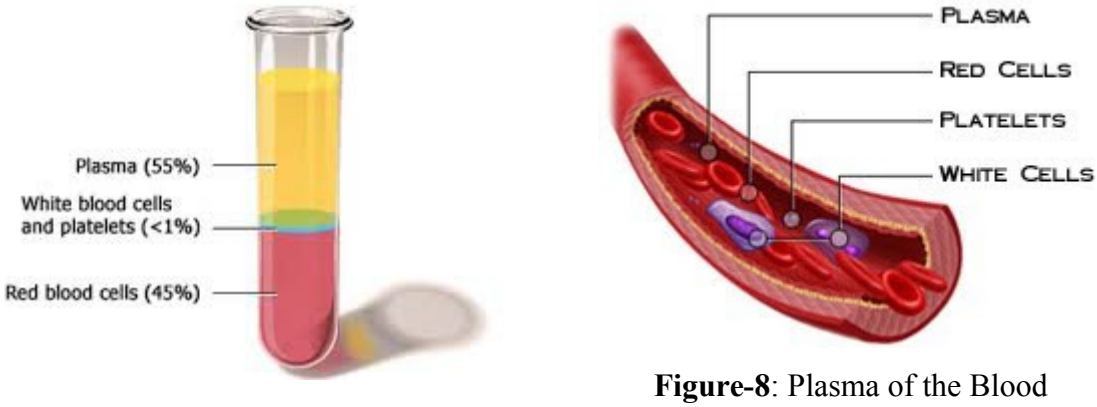


Figure-8: Plasma of the Blood

1.5.4 Cellular Elements

1.5.4.1 Red Blood Cell

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

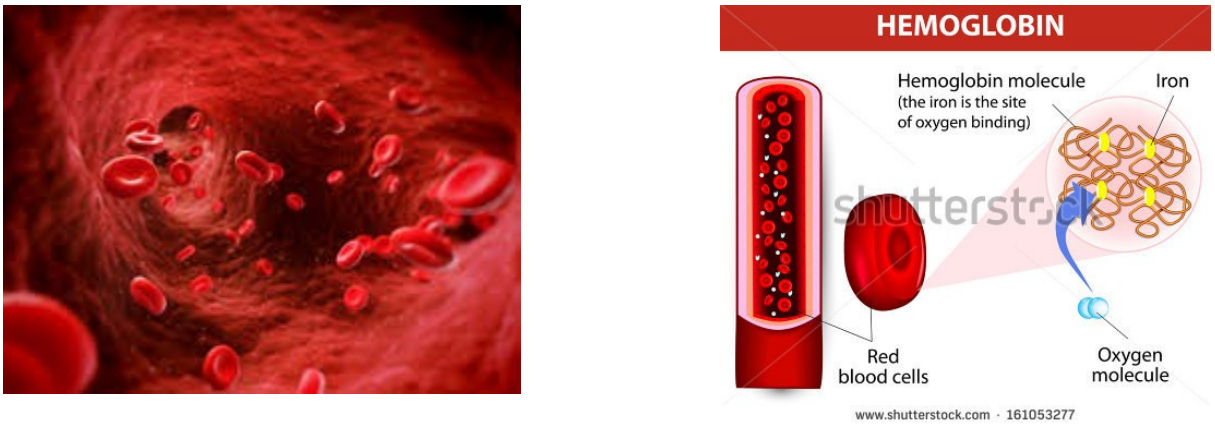


Figure-9: Red Blood Cell & Hemoglobin

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, 2004).

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

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

Higher than Normal MCV

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

Lower than Normal MCV

- The most common causes of microcytic anemia are iron deficiency (due to inadequate dietary intake, gastrointestinal blood loss, or menstrual blood

loss), thalassemia, sideroblastic anemia or chronic disease. In iron deficiency anemia (microcytic anemia), it can be as low as 60 to 70 femtolitres.

- In some cases of thalassemia, the MCV may be low even though the patient is not iron deficient (Tonnesen, 1986).

Mean corpuscular hemoglobin (MCH)

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

Higher than Normal MCH

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

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

Lower than Normal MCV

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

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

Mean corpuscular hemoglobin concentration (MCHC)

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

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

Normal range: 32-36 g/dL

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

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

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

Higher than Normal RDW

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

1.5.4.2 White Blood Cell

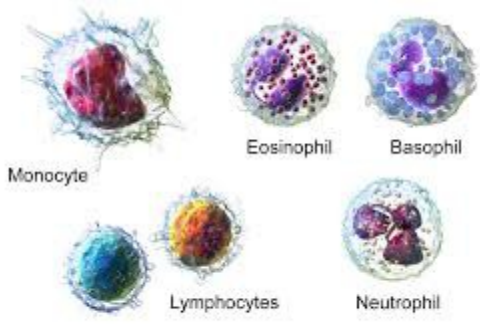
White blood cells are different from red cells in the fact that they are usually larger in size 10-14 micrometers in diameter. White blood cells do not contain hemoglobin which in turn makes them

translucent. Many times in diagrams or pictures white blood cells are represented in a blue color, mainly because blue is the color of the stain used to see the cells. White blood cells also have nucleii, that are some what segmented and are surrounded by electrons inside the membrane. White blood cells (leukocytes) are also known as "WBC's". White blood cells are made in the bone marrow but they also divide in the blood and lymphatic systems. They are commonly amoeboid (cells that move or feed by means of temporary projections, called pseudopods (false feet), and escape the circulatory system through the capillary beds. Normal range of WBC: 3-7×103mm3.

There are two types of WBC:

- ✓ Granular leukocytes: different types of granular WBC's are

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



White Blood Cells

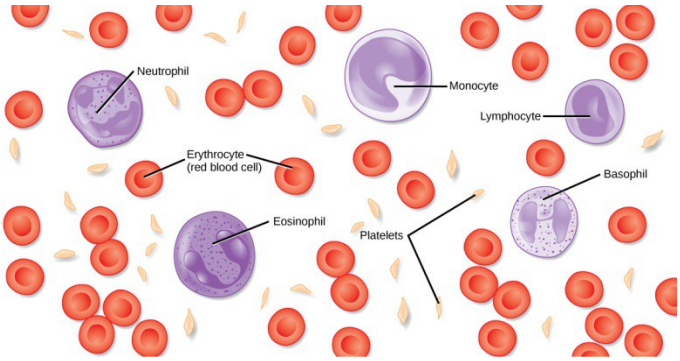


Figure-10: Different Parts of White Blood Cell and Platelet

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

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

Several factors predispose the liver to xenobiotic toxicity.

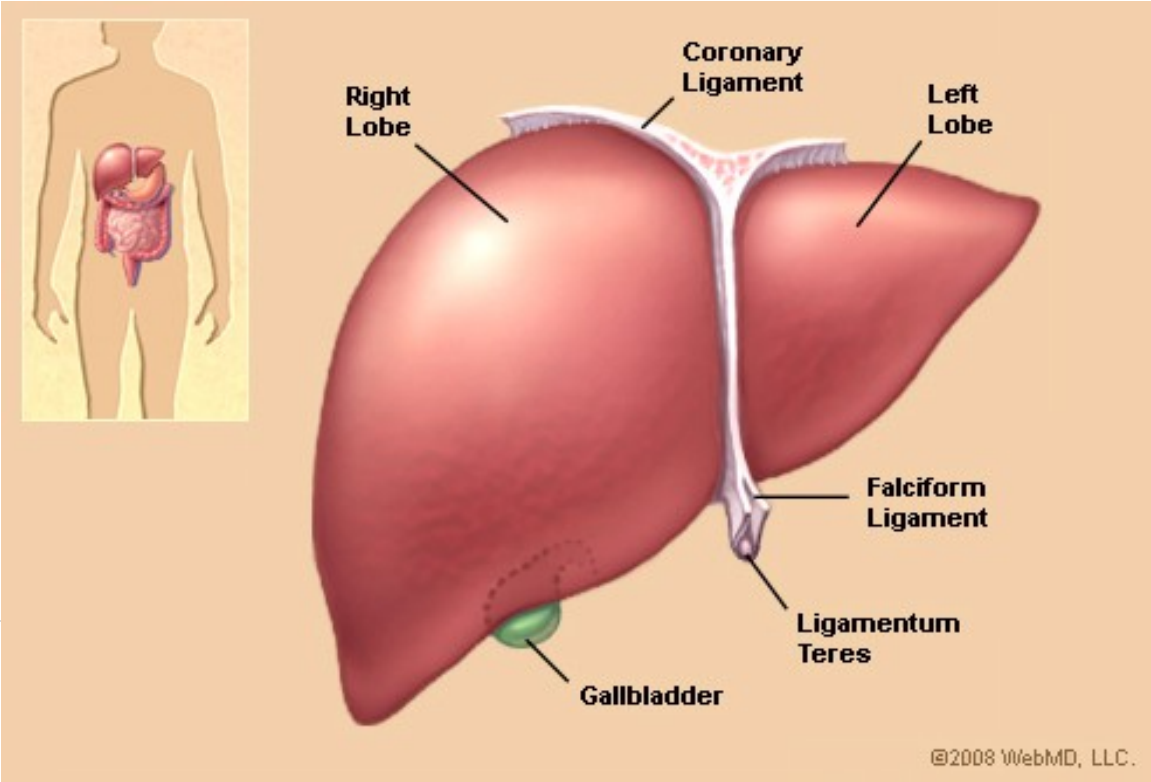
- Firstly, for chemicals entering the body via the oral route, anatomical proximity to the GI-tract ensures the liver is the 'first port of call' for ingested xenobiotics.
- Secondly, chemicals and nutrients are not the only substances that enter portal blood as it perfuses the intestines: it also accumulates products of the degradation of intestinal

microorganisms such as inflammogenic lipopolysaccharide components of the bacterial cell wall (i.e. endotoxin). Since endotoxin delivery may increase during xenobiotic intoxication, immunological responses to co-absorbed endotoxin can exacerbate the hepato-toxicity of ingested chemicals.

- Thirdly, in addition to entry via the portal circulation, chemicals can access the liver via arterial blood that mixes with venous blood in the hepatic sinusoids. For example, inhaled tobacco constituents that enter via the lungs are efficiently delivered to the liver via the arterial route.
- Fourthly, the vast metabolic capacities of the liver also paradoxically heighten its vulnerability to chemical toxicity: by functioning as a miniaturised chemical factory that performs many diverse chemical modifications on foreign molecules, CYPs and other hepatic enzymes can inadvertently generate noxious metabolites that induce 'bioactivation-dependent' hepatotoxicity (Philip, and Burcham, 2014).

1.6.1 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.

Figure-11: Anatomy of liver

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

Function

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

1.6.2 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. (Mc.Clatchey, 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, 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

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, et.al. 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, 2011).

1.6.2.3 Aspartate transaminase

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

An increase in ALT levels may be due to:

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

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). (Pratt, 2010).

2.1 Introduction of Plant, *Mikania cordata*

The plant, *Mikania cordata* (Bum.f) B.L. Robinson, is well known medicinal plant amongst traditional practitioner in India, Bangladesh, Brazil and Philiphines for its medicinal values that treat several local illness (Patar, AA. and Yahaya, BH., 2012).

Figure-12: Whole Plant of *Mikania cordata*

2.1.1. Description of *Mikania cordata:*

2.1.1.1. Scientific name: *Mikania cordata* (Burm. F.) Robinson

2.1.1.2. Local Name:

- ✓ **Indian Subcontinent:** Assamlata, Germanlata and Taralata
- ✓ **English:** Heartleaf hempvine, Mile a minute.
- ✓ **Philippines**: Bikas.
- ✓ **Chinese:** jia ze lan
- ✓ **French: liane marzoge, liane Pauline, liane raisin**

2.1.1.3. Taxonomic position

Kingdom: Plantae

Division: Magnoliophyta

Class: Magnoliopsida,

Order: Asterales

Family: Asteraceae

Genus: Mikania

Species: *Mikania cordata*

2.1.1.4. Description

A fast growing, creeping or twining, perennial vine; stems branched, pubescent to glabrous, ribbed, from 3 to 6 m long; leaves opposite, cordate or triangular-ovate, blade 3 to 12 cm long, 2 to 6 cm wide, on a slender petiole 1 to 8 cm long, base broadly cordate, tip acuminate, margins crenate, dentate, or entire, surfaces nearly glabrous, three- to seven-veined from base; flowers in small heads in open, nearly flat-topped (corymbose) panicles; axillary and terminal heads 6 to 9 mm long, four-flowered; involucral bracts four, obtuse or acute, 5 to 6 mm long, glabrous or subglabrous with one additional smaller bract about 3 mm long; corolla white or yellowish white, about 5 mm long; anthers bluish gray or grayish black; style white; fruit an achene, linear-oblong, 2 to 3 mm long, five angled, blackish brown, glandular; pappus of 40 to 45 bristles, about 4 mm long, white at first, reddish afterwards. May be distinguished by the following characteristics: 40 to 45 reddish pappus bristles, corollas white, and heads 7 to 7.5 mm long (Holm, *et al*. 1977).

2.1.2. Habitat/ecology

Grows most frequently in places receiving high rainfall, probably 1,500 mm or more; prefers rich, damp soil; rarely grows in dry areas; and thrives in open, disturbed places. For that reason it is common in young secondary forests, in forest clearings, in plantation tree crops, fallow or neglected lands, and along rivers and streams, waste areas, steep hillsides, and even mountainsides from whence winds probably spread the seeds to new areas. The species will grow in partial shade, but cannot tolerate dense shade" (Holm et al., 1977).

2.1.3. Geographical Distribution

Mikania (Asteraceae) species are found throughout tropical regions of Africa, Asia (Bangladesh, India), Brazil and South America (Argentina, Paraguay and Uruguay) *Mikania* is native to Central and South America, and has become a serious weed in West Africa through to India, South-East Asia, Indonesia and the Pacific Islands. *Mikania* was first found in Australia in 1998 at Ingham and Bingil Bay, and has since been detected at one location near Speewah, near Mareeba (Chowdhury, et.al. 2011).

2.1.4. Chemical composition

Different classes of compounds were previously isolated from various *Mikania* parts, which can be associated to this plant's pharmacological activities. The main groups are: coumarins and derivatives, sesquiterpenes, sesquiterpenes lactones, diterpenes, phytosterols/terpenoids and flavonoids. Caffeoylquinic acid derivatives beyond others chemical compounds are found in smaller amount. Diterpenes such as kaurenoic acid and benzoylgrandifloric acid (class of kauranes), have also attracted interest for their pharmacological action. Moreover, detailed screenings revealed the presence of other substances in species of *Mikania* as alcohols, acids, esters, aldehydes and organic esters (Gasparetto et al., 2010).

- **Coumarins and derivatives**

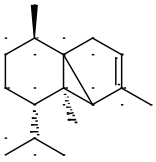
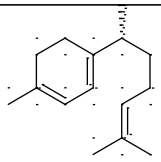
The most characteristic class of compounds in *Mikania* genus is the coumarins and derivatives, frequently responsible for pharmacological activity. A wide variety of biological activities is assigned for these compounds, such as antimicrobial, antiviral, anti-inflammatory, antispasmodic, antitumoral, anticoagulant, bronchodilator and antioxidant (Pereira et al., 1994; Hoult & Payá, 1996). The coumarin (1,2-benzopyran), dihydrocoumarin and o-coumaric acid

scopoletin, O-geranylscopoletin herniarin (7-methoxycoumarin) and 2,6-dimethoxyquinone were identified in extracts of Mikania genus (Vidal et al., 2006).

- **Sesquiterpenes and terpenes, diterpenes and sesquiterpenes lactones**

Sesquiterpenes are abundant in Mikania genus, related to that the most commom are germacrene D, isocomene and γ-humulene. These compounds were reported in around 15% of Mikania species that already had their chemical composition determined, (Bohlmann et al., 1982). Likewise, terpenes, diterpenes and sesquiterpene lactones are often found, mainly the lactones type mikanolide and miscandenin derivatives, which have analgesic activity (Ahmed et al., 2001), antibacterial (Facey et al., 2010) and anticancer properties (Prevost et al., 2002).

Table-4: Different isolated compound in different parts of *Mikania Cordata*

Plant specie	Geographic Distribution	Part used	Compounds	Structure
M. cordata	Asia	Leaves	• α-cubebene (21.3%) , • caryophyllene oxide (10.1%), • α-bisabolol (6.6%), • γ-curcumene (6.3%), • β-pinene (4.1%), • copaene (4.1%), • α-cedrene (4.9%), • spathulenol (3%)	 α-cubebene
M. cordata	Asia	flowers oil	• β-pinene (14.9%), • α-cubebene (12.4%), • γ-curcumene (11.7%) • aryophyllene (8.5%), • α-bergamotene (5.6%), • β-caryophyllene(4.3%), • zingiberene (6%)	 γ-curcumene

- **Diterpenes**

Some diterpenes are common in *Mikania* genus like kaurenoic acid, which is characterized by its trypanocidal activity. Also, the kaurenoic acid has other important activities such as antimicrobial, antinociceptive, anti-inflammatory and smooth muscle relaxant (Gasparetto et al., 2012). In a study performed by Nunez et al. (2004) on leaves of *Mikania* sp. nov., found in the state of Bahia, Brazil, several diterpenes were obtained: labda-8(17),12,14-trien-19-oic methyl ester, pimara-9(11),15-dien-19-oic methyl ester, labda-8(17),13(16),14-trien-19-oic methyl ester, labda- 12α-epoxy-8(17),14-dien-19-oic methyl ester, labda- 12β-epoxy-8(17),14-dien-19-oic methyl ester, erythroxyla-3,15-dien-19-oic acid, labda-12,15- epoxy-8(17),13-dien-19-oic acid, and labda-12,13-dihydroxy-8(17),14-dien-19-oic methyl ester.

- **Phytosterols/terpenoids**

The most common phytosterols present in approximately 10% of species of *Mikania*, that has its chemical composition determined, are stigmasterol, lupeol and sitosterol. These compounds have been detected in the aerial parts and are found in the species *M. cordata* (Aguinaldo et al.,2003). The terpenoids amyrin and friedelin , abundant in *Mikania* genus, were reported in *M. micrantha*, *M. cordata*, *M. cordifolia* (Oliveira et al., 2006).

- **Flavonoids**

Flavonoids are popular due to their antioxidant activity and are widely present in *Mikania* genus supporting its pharmacological activity. In *M. cordata*, flavonoids were described as patuletine-3-*O*-β-D-6"-(p-coumaroyl), glucoside(6-methoxyquercetin-3-*O*- β -D-6"-(*p*coumaroyl) glucoside), mikanin-3-*O*-sulfate (salt as Ca+2), eupalitin-3-*O*-sulphate (as salt K+), eupalitin- 3-*O*-β-D-glucoside, 6-methoxykaempherol-3-*O*-β- D-glucoside, nepetin and kaempherol-3-*O*-α-L-rhamnoside. For the same species, it was reported the isolation of a flavone, mikanin-(3,5-dihydroxy-4',6,7-trimethoxyflavone) with epifriedelinol from roots and fumaric acid from leaves and stems (Aguinaldo et al., 2003).

- **Caffeoylquinic acid and derivatives**

The chemical compound 5-caffeoylquinic acid is a caffeic acid ester, also known as a chlorogenic acid, commonly found in a wide number of plants, *e.g*. coffee. It is produced in plants via an ester bond between the carboxyl group of caffeic acid and the 5-hydroxyl group of

quinic acid (Clifford et al., 2006). The chlorogenic acid and caffeic acid were reported as dampening the risk of chronic diseases such as inflammation, cardiovascular diseases and cancer (Boyer & Liu, 2004; Bonita et al., 2007).

2.1.5. Pharmacological activities

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

- **Activity in the respiratory tract**

Medicinal plants play an important role in maintaining public health, mainly due to their low cost and availability. Some plants acting in the respiratory system, such as *Mikania* genus, have confirmed their effectiveness. For example, *M. glomerata*, one of the most important and commonly used species *Mikania* genus, has been popularly used in the treatment of asthma, bronchitis and coughing (Agra et al., 2008). Other species, known as "guaco" are also used to treat respiratory problems as *M. cordifolia* (Oliveira et al., 2006), *M. laevigata,* and *M. cordata* (Ali et al., 2011).

- **Activity in the digestive system**

Many plants and their extracts are commonly used for acting against several disorders of the digestive system. Among them are some species of the genus *Mikania* as *M. glomerata*, *M. laevigata* and *M. cordata.* Moreover, the decoction of the leaves of *M. cordata* also shows effects in the digestive system. It is used in dyspepsia, dysentery and gastric ulcer (Ghani, 1998). The methanolic fraction of root extract showed antiulcer effects in male Sprague-Dawley rats in a dose dependently manner inhibiting gastric ulcers induced by water immersion stress-induced, ethanol, aspirin and phenylbutazone.

- **Effect on nervous system**

Mikania extracts possesses some neuropharmacological properties confirmed. The studies with methanolic fraction of *M. cordata* root extract on experimental animals caused alterations in the general behavior pattern (*e.g.* reduction in spontaneous motility, analgesia, and suppression of aggressive behaviour), suppression of conditioned avoidance response and showed antagonism to amphetamine toxicity. The observations suggest that the root of *M. cordata* possesses a potent central nervous system depressant action (Bhattacharya, et al. 1988).

- **Anti-inflammatory, anti-allergic and analgesic activity**

The inflammatory response is associated with a range of diseases and it is difficult to establish an effective therapy to control the inflammatory processes. So, there is a clear and obvious need to search for new medicinal compounds, especially those derived from plants. Studies with extracts, oils and compounds of several species have demonstrated their important activity. The compound scandenolide, a sesquiterpene lactone present in *M. cordata*, exhibited anti-inflammatory activity. It also inhibited the production of leukotriene B4 and 5-HETE with IC50 of 15 and 30 μM concentration, respectively (Ysrael & Croft, 1990). In addition, the crude extract of *M. cordata* (1 and 3 g/kg) and a sesquiterpene lactone deoxymikanolide (10 mg/kg) significantly inhibited acetic-acid induced writhing in mice (Ahmaed et al., 2001).

- **Antimicrobial, antivirucidal and antiparasitic activity**

The antimicrobial and antiparasitic properties of compounds present in plants as products of secondary metabolism have been known empirically for centuries, but only recently they have been scientifically confirmed. Extracts and essential oils from plants proved their efficacy in controlling the growth of a wide variety of microorganisms, including bacteria, fungi, parasites and others. In a study carried out with hexanic extract of *M. cordata*, it was observed the inhibition growth of a multiresistant strain of *Staphylococcus aureus* PI57, verified by antibiogram and bioautography.

- **Antiophidic activity**

Although serotherapy was discovered one hundred years ago, many rural communities do not have access to antivenoms. In this way, they alternatively use plants with antiophidic activity known in popular culture, such as some species of the genus *Mikania*. The extract components

were effective in mammals to inhibit the letal effects of poisonous animals, such as nauyaque snake and rattlesnake, scorpions, spiders and bees. The author has indicated its use for treating snake bites, scorpion stinging, bee sting and similar.

- **Antimutagenic and cytotoxic activity**

The natural products provide very important chemical libraries that have led to new antimutagenic drugs (Cragg et al. 2009). The chemopreventive role of *M. cordata*, was evaluated for its effects on phase 1 and 2 of the hepatic drug detoxifying enzyme system in rats (Bishayee & Chatterjee, 1994). In oral doses of 50, 100, or 150 mg/kg of extract for 4, 8 or 12 weeks results in dose dependent effects on a marked induction of uridine diphosphoglucuronyl transferase activities of liver microsomes and others effects. The study indicated that the carcinogens would be reduced by specific enhancement of drug-detoxifying enzymes in the liver of rats treated with the plant extract.

- **Allelopathic activity**

Allelopathy is defined as any indirect or direct, beneficial or damaging effect, from a plant to other, resulted from the production of chemical products which are released into the environment. The same chemical compounds responsible for the allelopathic activity can be modulated in some pharmacological activity. It has also attracted great interest due to their potential applications in agriculture and therefore has been studied in several plants.

2.2 Introduction of plant, *Spilanthes acmella*

Spilanthes acmella, a well-known antitoothache plant with high medicinal usages, has been recognized as an important medicinal plant and has an increasingly high demand worldwide. From its traditional uses in health care and food, extensive phytochemical studies have been reported.

Figure-13: Whole plant of *Spilanthes acmella*

2.2.1. Description of *Spilanthes acmella*

2.2.1.1. Scientific name: *Spilanthes acmella*

2.2.1.2. Local Name: Marhatitiga

2.2.1.3. Taxonomic position

Kingdom: Plantae

Subkingdom: Tracheobionta

Superdivision: Spermatophyta

Division: Magnoliophyta

Class: Magnoliopsida

Subclass: Asteridae

Order: Asterales

Family: Asteraceae

Genus: *Spilanthes*

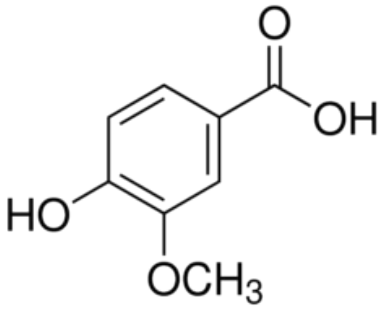
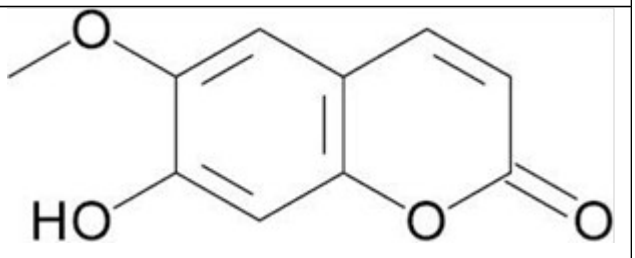
Species: *Spilanthes acmella*

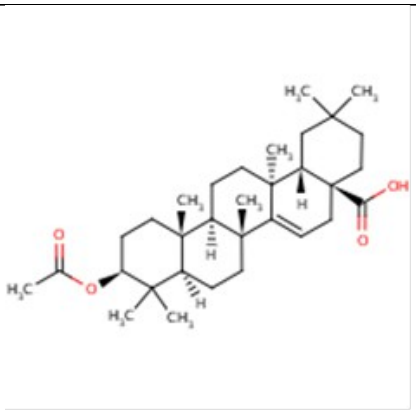
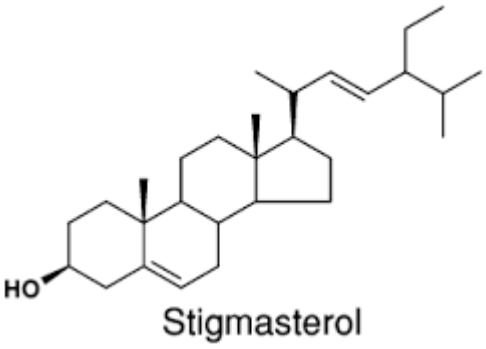
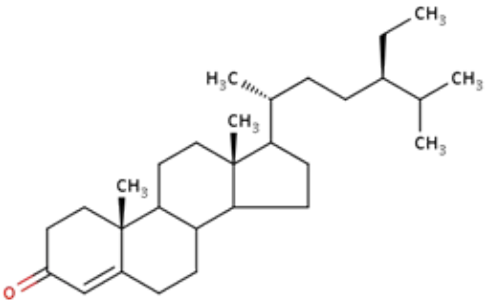
2.2.1.4. Description

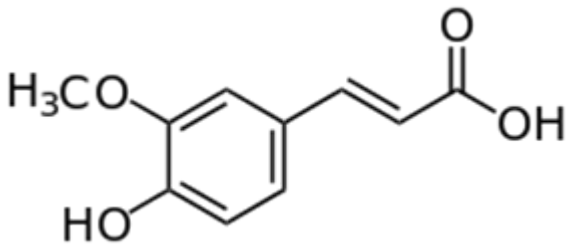
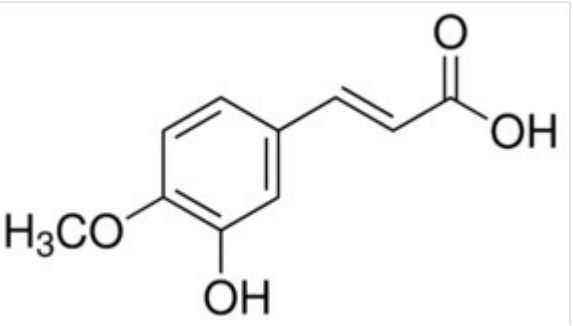
Spilanthes (Compositae or Asteraceae) is a genus comprising of over 60 species that are widely distributed in tropical and subtropical regions of the world, such as Africa, America, Borneo, India, Sri Lanka and Asia (Sahu et al., 2011; Tiwari et al., 2011). *S. acmella* is native to Brazil and is cultivated throughout the year as ornamental or medicinal plant. It is an annual or short-lived herb that is 40-60 centimeters tall. It is grown in damp area (Tiwari et al., 2011; Wongsawatkul et al., 2008) and has low rate of germination or poor vegetative propagation (Tiwari et al., 2011). Its flowers and leaves have pungent taste and when touched it is accompanied by tingling sensation and numbness (Wongsawatkul et al., 2008). The plant species has been used commonly as a folk remedy, e.g. for toothache, rheumatic and fever (Wongsawatkul et al., 2008), as fresh vegetable (Tiwari et al., 2011) as well as spice for Japanese appetizer (Leng et al., 2011).

2.2.2. Chemical composition:

Table-5: Bioassay-guided isolation resulted in a diverse group of bioactive compounds of *Spilanthes acmella*

Type	Compound	Structure
Phenolics	vanillic acid, *trans*-ferulic acid and *trans*isoferulic acid	 vanillic acid
Coumarin	Scopoletin	 Scopoletin

Triterpenoids	3-acetylaleuritolic acid, β-sitostenone, stigmasterol and stigmasteryl-3-*O*-β-D-glucopyranosides, in addition to a mixture of stigmasteryl-and β-sitosteryl-3-*O*-β-D-glucopyranosides.	 β-sitostenon
Hexane extract	Stigmasterol	 Stigmasterol
Chloroform extract	stigmasterol, stigmasteryl-3-*O*-β-D-glucopyranoside	
Ethyl acetate extract	3-acetylaleuritolic acid, vanillic acid and β-sitostenone	 3-acetylaleuritolic acid

Methanol extract	scopoletin, *trans*-ferulic acid, *trans*-isoferulic acid and a mixture of stigmasteryl-3-*O*-β-D-glucopyranoside and β-sitosteryl-3-*O*-β-D-glucopyranoside (MBSG).	 *trans*-ferulic acid  *trans*-isoferulic acid

2.2.3. Bioactivity

The *Spilanthes* genera have been used for the treatment of various disorders including life-threatening diseases. Diverse pharmacological activities of this plant species were previously reported (Sahu et al., 2011; Tiwari et al., 2011). Selected bioactivities of *S. acmella* are summarized below.

- **Antipyretic activity**

Many medicinal plants have long been used as antipyretics, e.g. *S. acmella* (flower and aerial aqueous) extracts (Chakraborty et al., 2010). *S. acmella* (aerial aqueous extract) displayed antipyretic activity against Brewer's yeast induced pyrexia. The antipyretic activity of the plant species can be attributed to flavonoids (Narayana et al., 2001); which were predominant inhibitors of either cyclooxygenase (COX) or lipoxygenase (LOX) (Sadavongvivad and Supavilai, 1977). Flavonoids are known to target prostaglandins in the late phase of acute inflammation and pain (Chakraborty et al., 2004).

- **Antiinflammatory activity**

Spilanthol is the main constituent isolated from many parts of *S. acmella* such as flower 85% EtOH extract(Wu et al., 2008), root hexane extract (Wagner, 1989). Traditional usages of *S. acmella* flowers have been reported as anti-inflammatory agent (Sharma, 2003). Moreover, EtOH extract from the leaves of *S. acmella* exhibited significant antiinflammatory activity against acute (carragenan induced rat paw edema method), sub-acute (granuloma pouch method) and chronic (adjuvant arthritis method) inflammation (Barman et al., 2009) but has been shown to be less than that of aspirin. The observed anti-inflammatory activity originates from the inherent flavonoids that are found in the plant extracts (Chakraborty et al., 2004).

- **Local anesthetic activity**

S. acmella is known to be constituted of pungent alkamide-like spilanthol that causes numbness and tingle. *S. acmella* aerial aqueous extract exhibited significant activity that could be due to the presence of alkamides (Chakraborty et al., 2010). However, its onset of action was slower than that of xylocaine, the standard drug. Its mechanism of action involves the blockage of voltage-gated Na+ channels. By the same analogy, the alkamides of *S. acmella* extracts produced local anesthetic action presumably through the blockage of Na^+ channels.

- **Antimicrobial activity**

Ethyl acetate (EtOAc) and methanol (MeOH) extracts from the leaves of *S. acmella* exhibited the strongest antimicrobial activity among the tested extracts using the well diffusion method against *Klebsiella pneumonia*e (Arora *et al*., 2011). The EtOAc extract had two-fold higher activity than that of doxycycline, the standard drug, whereas the MeOH extract showed comparable activity with doxycycline. This could be due to the fact that the plants contain flavonoids, tannins, and other phytochemicals, which are well-known antimicrobials.

- **Antimalarial activity**

S. acmella is a traditional medicine used in Africa and India for the treatment of malaria (Spelman et al. 2011). spilanthol and acetylenic alkamide (undeca-2*E*-ene-8,10- diynoic acid isobutylamide or UDA), isolated from the root EtOH extract of *S. acmella*, displayed antimalarial activity against two strains of *Plasmodium falciparum.*

- **Antioxidant activity**

Antioxidant activity of *S. acmella* extracts obtained from polar and nonpolar solvents were investigated. It was found that *S. acmella* flower EtOAc extract displayed the highest free radical scavenging activity when compared to the other tested extracts (Wu *et al*., 2008). On the other hand, leaves and flowers of *S. acmella* MeOH extracts showed weak antioxidant activity (Nanasombat and Teckchuen, 2009).

- **Diuretic activity**

The study of *S. acmella* EtOH leaves extract revealed diuretic effect possibly arising from tannin, steroid and carotenoid (Vanamala et al., 2012). In addition, flower cold aqueous extract of the plant species exhibited strong diuretic activity (Kumar et al., 2010). The effect may be attributed to its alkaloids. It was suggested that the extract acted as a loop diuretic, which is the most powerful of all diuretics (Ratnasooriya et al., 2004).

3.1 Plant Preparation

3.1.1 Collection of plant

The plant was collected from Foridpur district of Bangladesh. A voucher specimen (Accession number: 38479) had been deposited at the Bangladesh National Herbarium. The proper time of harvesting or collecting is particularly important because the nature and the quantity of constituents very gently in some species according to the season.

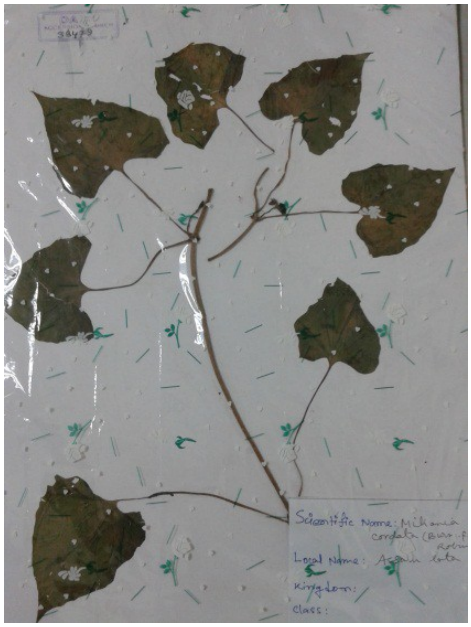


Figure-14: Herbarium sheet of *Mikania cordata*

3.1.2 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 2kg dried plant dust of *Mikania cordata* was soaked in L methanol in four bottles. Then it was kept in room temperature for 3 days and everyday it was used to shake properly to ensure the maximum amount of constituents present in the grinded plant become soluble into methanol. After 3 days later, the mixture was filtered. For filtration, white cotton cloth was used. After filtration two parts were obtained.

1. The residue portion over the filter

2. The filtered part

The filtrated part, which contains the substance soluble in methanol, poured into a 1000 round bottle flask, and then the flask was placed in a rotary evaporator. The evaporation was done at 53 degree Celsius temperature. The number of rotation per minute was selected as 125 RPM. The pressure of vacuum pump machine was 6 bars. The water flow through the distillation chamber was also provided in a satisfactory flow rate.

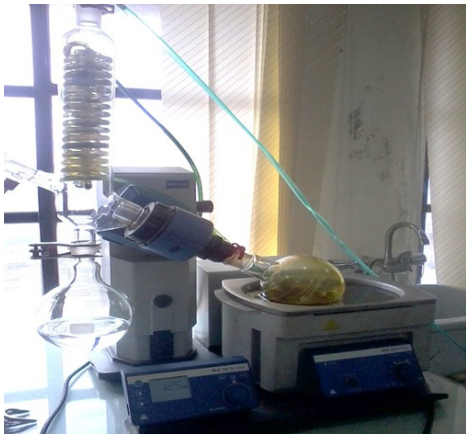


Figure-15: Rotary evaporator & crude extract in a bottle

3.1.3 Crystal formation

After completing rotary crystal formation was occurred that was good in amount. These crystals are clear and stable. These crystals are not soluble in polar and not polar solvent and intermediate solvent. Further investigation will be continued to know about these crystals.

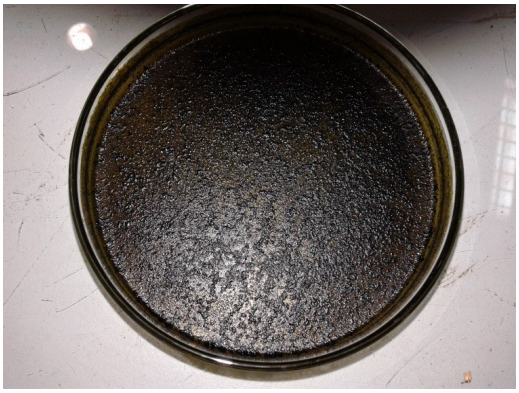


Figure-16: Formation of crystals from crude extract

3.1.4 Liquid-liquid Extraction

The crude extract was subjected to liquid-liquid extraction using three solvent systems, N-hexane, Dichloromethane (DCM) and water system. At first 55 gm of crude extract was mixed with distilled water and methanol. Then added N-hexane to the mixer and mixed properly. Then it poured into a separating funnel. After some time two layers was separated.

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

Then we collected the bottom layer slowly and subjected to evaporation to get N-hexane extract of *Mikania cordata.*

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

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

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

Figure-17: Fractions of *Mikania cordata*

3.2 Experimental Animals

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

Figure-18: *Swiss albino* Mice

3.3. CNS Activity Test

3.3.1. Materials for CNS Activity Test:

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

3.3.2. Chemical Agents Used in Analgesic activity Test:

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

3.3.3. Standard Drugs Used in CNS activity Test:

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

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

i) Open Field Test:

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

ii) Hole Board Test:

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

3.3.5. Methods for CNS Activity Test:

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

- Open Field Test.
- Hole Board Test.

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

Open Field Test:

In this experiment, the method according to Gupta, 1971 was employed. An open field, a test paradigm which is highly standardized to evaluate locomotor activity (Kelley, 1993). The animals were divided into negative control, positive control and test groups containing six mice in each group. Negative control group received vehicle (5% CMC solution) at a dose of 10 mg/kg body weight orally. The test groups received extracts of *Mikania cordata* at the doses of 200 and 400 mg/kg body weight orally. The floor of an open field of half square meter was divided in to a series of squares, each alternatively colored black and white. It has 49 squares. The number of Peripheral locomotion (movement of mice on surrounding 40 squares other than central 9 squares), number of Central locomotion (movement of mice on central 9 squares), number of Leaning (standing of mice with the help of wall) and number of Rearing (standing of

mice without any help) number of Grooming (face rubbing or itching), and number of defecation was recorded for a period of two minutes. The observation was conducted at 0, 30, 60, 90 and 120 minutes after oral administration of test drugs and was compared with control animal.

Figure-19: Open Field Test

Hole Board Test

The hole board represents a combination of a hole board, originally designed to investigate explorative motivation in rodents (Lister, 1990) and later on modified to evaluate cognitive functions (Ohl and Fuchs, 1999; Ohl et al., 1998) The hole board itself consisted of a total of 16 holes, each 3 cm in diameter, were presented to the mouse in a flat space of 25 square centimeters. This experiment was carried out by the following method of Boisser and Simon, (1964). The animals were divided into negative control and test groups containing six mice in each group. Negative control group received vehicle (5% CMC solution) at a dose of 10 mg/kg body weight orally. The test groups received extracts *Mikania cordata* at the doses of 200 and 400mg/kg body weight orally. Each of the animal was transferred carefully to one corner of the field and the number of ambulation (expressed as the number of holes passed), head dipping and number of head poking was recorded for a period of 5 minutes at and post 30 minutes intervals and were compared with the control animals

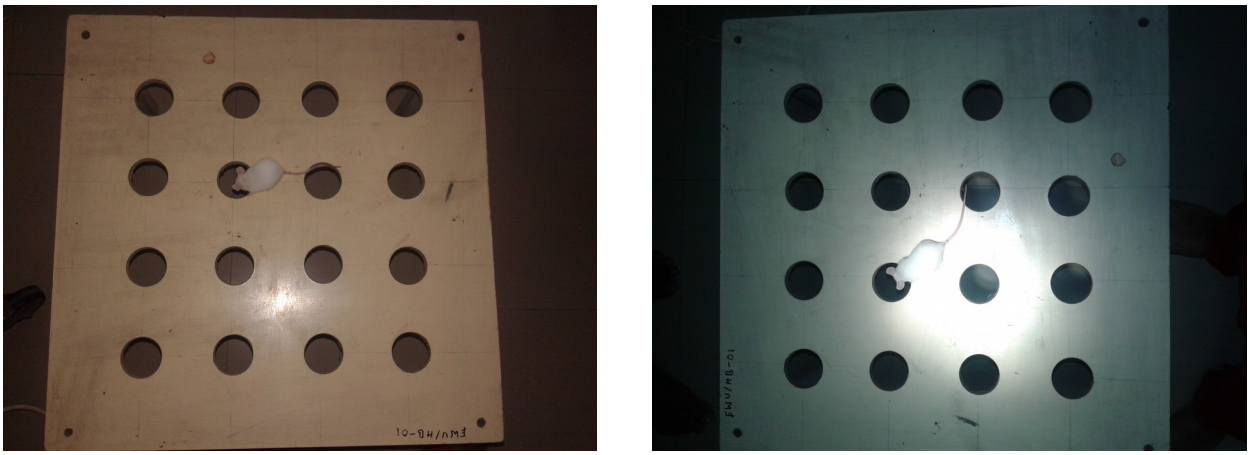


Figure-20: Hole Board Test

3.4. Analgesic Activity Test:

3.4.1. Materials for Analgesic Activity Test:

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

3.4.2. Chemical Agents Used in Analgesic activity Test:

- 5% CMC (Vehicle) 10ml/kg as negative control,
- 0.7% v/v, Acetic acid in 0.9% saline (10ml/kg).
- 5% Formalin in 0.9% saline (20µL).

3.4.3. Standard Drugs Used in Analgesic activity Test :

- Indomethacin (10mg/kg) used as positive control in acetic acid induced writhing test.
- Aspirin (100mg/kg) used as positive control in formalin induced paw licking test.

3.4.4. Doses Used in Analgesic Activity Test of the Extract:

i) Acetic Acid induced Writhing Test:

- Methanolic extracts of *Mikania cordata* at a dose of 800mg/kg, 1200mg/kg of the crude extract and fraction of N-Hexane, DCM (Dichloromethane) at a dose of 500 mg/kg are administered orally. Also fraction of n-Hexane, chloroform, ethyl acetate and water fraction of *Spillanthus acmella* at a dose of 200mg/kg are administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses.

ii) Formalin Induced Paw licking Test:

- Methanolic extracts of *Mikania cordata* at a dose of 800mg/kg, 1200mg/kg of the crude extract and fraction of N-Hexane, DCM (Dichloromethane) at a dose of 500 mg/kg are administered orally. Also fraction of n-Hexane, chloroform fraction of *Spillanthus acmella* at a dose of 200mg/kg are administered orally. 5% CMC was used as a vehicle with plant methanolic extract for preparing different doses.

3.4.5. Methods for Analgesic Activity Test:

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

- Acetic Acid Induced Writhing Test.
- Formalin Induced Paw licking Test.

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

Acetic Acid Induced Writhing method:

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

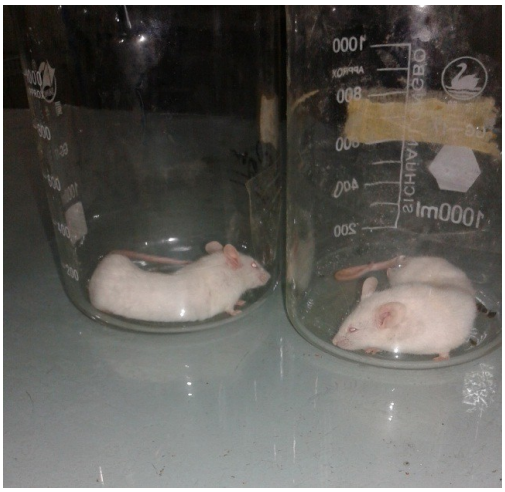


Figure-21: Writhing of mice

The analgesic activity of samples are evaluated using acetic acid induced writhing test. At first *Swiss albino* mice of either sex are divided into 6 groups , named group-1, group-2, group-3, group-4. Each group contains 6 mice (n=6). After an over night fast, 5% CMC is given orally (at dose 10ml/kg) to group-1(considered as negative control). Then crude plant extract (suspended in 5% CMC vehicle) of *Mikania cordata* is given orally to group-2, group-3 at dose 800mg/kg& 1200mg/kg (as per body wt of mice) and fraction of n-hexane, DCM of of *Mikania cordata* is given orally to group-4, group-5 at dose 500mg/kg& 500mg/kg respectively. Again fraction of n-Hexane, chloroform, ethyl acetate and water of *Spillanthus acmella* at a dose of 200mg/kg are administered orally to group-2, group-3, group-4, group-5. After 30 min of oral drug administration, 0.7% v/v acetic acid solution (Dose: 0.1ml/10gm as per body wt of mice) is injected intraperitonially to the all groups.

Figure-22: Process of Intra-peritoneal injection to mice.

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

It is calculated as:

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

where, W_c = number of writhing of the control group

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

Formalin Induced Paw Licking Method:

Following an overnight fast, the Mice are divided into 6 groups of mice. 5% CMC (negative control) at a dose of 10ml/kg is given orally. Then crude plant extract (suspended in 5% CMC vehicle) of *Mikania cordata* is given orally to group-2, group-3 at dose 800mg/kg& 1200mg/kg (as per body wt of mice) and fraction of n-hexane, DCM of *Mikania cordata* is given orally to group-4, group-5 at dose 500mg/kg& 500mg/kg respectively. Again fraction of n-Hexane, chloroform, ethyl acetate and water of *Spillanthus acmella* at a dose of 200mg/kg are administered orally to group-2, group-3, group-4, and group-5. Each group contains 6 mice (n=6). After 30 min of oral drug administration, 20µl of 5% v/v formalin solution (5ml of formalin in 95 ml of 0.9%Nacl solution) is injected subcutaneously to the treatment groups of mice to their left hind paw of the mice. Simultaneously, 100mg/kg Aspirin is administered to animals in a group, Thirty minutes after treatment, 20 µl of 5% formalin was injected subcutaneously into the left hind paw of the mice. Then licking time (in sec) is counted for observation. 1st phase (0-5 min after formalin injection) is anti-nociceptive phase & last phase (15-30 min after formalin injection) is considered as anti- inflammatory phase. Percentage inhibition of formalin induced paw licking time is also calculated

Figure-23: Oral administration into mouse.

It is calculated as: $\{(t_c - t_t) \times 100\}/t_c$

where, t_c = formalin induced paw licking time of the control group, t_t = formalin induced paw licking time of the treated group (Chang et al.,2012).

3.5. Toxicity Test

3.5.1 Materials for Toxicity Test

- Analytical Balance,
- Feeding needle: 1 c.c.
- Insulin syringes 100 units disposable
- 5 ml syringe disposable
- Dissecting box
- Dissecting pad
- Pin
- Beaker 1 litre
- Petri dish for washing
- Epindrop tube
- 250 ml food grade plastic pot
- Gloves
- Mask

3.5.2 Chemical Agents Used Toxicity Test

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

3.5.3 Doses Used for Toxicological Activity of the Extract:

i) Acute Toxicity Test:

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

ii) Sub-chronic Toxicity Test:

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

3.5.4 Methods for Analgesic Activity Test:

Acute Toxicity Test

The acute toxicity of in Swiss albino mice was studied as reported method. Each extract were given to three groups (n = 6) of mice at 2000 and 3000, 6000 mg/kg body weight, orally. The treated animals were kept under observation for 3 days, for mortality and general behaviour. (Paul, et.al. 2012).

Sub-chronic Toxicity Test

The adult Swiss albino mice were divided into four groups containing 4 animals per group. The first group received 5% CMC (Vehicle) 10ml/kg and the other three groups received the three doses of extracts like 200 mg/kg, 400 mg/kg, 600 mg/kg according to body weight orally,

respectively daily for 45 consecutive days. Food and water intake of animals were observed during this period. Body weight was taken for every 3 days. Twenty four hours after the last dose (i.e., at the 44th day), the mice were fainted by using chloroform and collected blood using 5 ml disposable syringe from cardiac puncture and reserved it in both heparinized and non-heparinized Epindrop tube. Then also collected other organ like Brain, Liver, Kidneys, Heart, Lung, and Stomach and reserved it food grade plastic pot having 5% formalin. Then this blood and liver was used for the study of Hematology test, Protein Test and Liver biochemical parameters Test (Paul, et.al. 2012).

3.5.5. Hematological parameters

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

3.5.6. 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.

3.5.7. 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 Haemotoxylin stain (Paul, et.al., 2012).

3.6. Statistical Analysis

Data obtained from pharmacological experiments are expressed as mean±SEM. Difference between the control and the treatments in these experiments were tested for significance using

one-way analysis of variance (ANOVA), followed by Dunnet's t-test for multiple comparisons using SPSS -16 software.

4.1 CNS Activity Test of Methanolic Extract of *Mikania cordata*

4.1.1. Open Field Test:

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

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

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

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

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

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

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

- ✓ The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 108.33±0.8***, at 30 min 96.16±1.62***, at 60 min 84.50±1.64***, at 90 min 75.67±1.14*** and at 120 min 67.50±0.99*** (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of central locomotion count is followed with a mean value of at 0 min 18.83±0.79***, at 30 min 14.0±0.96*** at 60 min 11.83±0.94***, at 90 min 8.67±0.67*** and at 120 min 6.50±0.76*** (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of leaning count is followed with a mean value of at 0 min 18.67±0.49***, at 30 min 15.16±0.70***, at 60 min 12.0±0.57***, at 90 min 9.50±0.56*** and at 120 min 7.5±0.76*** (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of rearing count is followed with a mean value of at 0 min 0.83±0.83**, at 30 min 1.67±1.05**at 60 min 2.00±1.12**, at 90 min 1.0±1.0** and at 120 min 0 (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of grooming count is followed with a mean value of at 0 min 3.0±0.73** at 30 min 1.67±0.76** at 60 min 0.33±0.33** at 90 min 0.83±0.54** and at 120 min 0(Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of defecation count is followed with a mean value of at 0 min 0.33±0.21** at 30 min 0, at 60 min 0, at 90 min 0.16±0.16** and at 120 min 0 (Mean ±SEM) during 2 minutes observation.

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

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

- ✓ The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 107.83±1.81***, at 30 min 84.50±1.47***, at 60 min 63.83±1.62***, at 90 min 53.83±1.70*** and at 120 min 43.50±1.56*** (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of central locomotion count is followed with a mean value of at 0 min 19.16±1.01***, at 30 min 15.33±0.88***, at 60 min 10.0±0.73***, at 90 min 7.16±0.60*** and at 120 min 4.83±0.60*** (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of leaning count is followed with a mean value of at 0 min 17.83±0.94***, at 30 min 13.0±0.73***, at 60 min 10.83±0.60***, at 90 min 6.67±0.49*** and at 120 min 4.83±0.30*** (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of rearing count is followed with a mean value of at 0 min 0, at 30 min 0, at 60 min 0.83±0.54**, at 90 min 0 and at 120 min 0.50±0.50** (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of grooming count is followed with a mean value of at 0 min 1.83±0.60** at 30 min 0.33±0.33** at 60 min 0 at 90 min 1.16±0.74** and at 120 min 0(Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of defecation count is followed with a mean value of at 0 min 0.50±0.22** at 30 min 0.33±0.21**, at 60 min 0.16±0.16**, at 90 min 0 and at 120 min 0 (Mean ±SEM) during 2 minutes observation.

Positive Control Group (Diazepam, 1mg/kg)

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

- ✓ The observed total number of peripheral locomotion count is followed with a mean value of at 0 min 111.67±1.3***, at 30 min 69.0±1.06***, at 60 min 50.0±1.54***, at 90 min 26.83±1.19*** and at 120 min 15.0±1.41*** (Mean ±SEM) during 2 minutes observation.

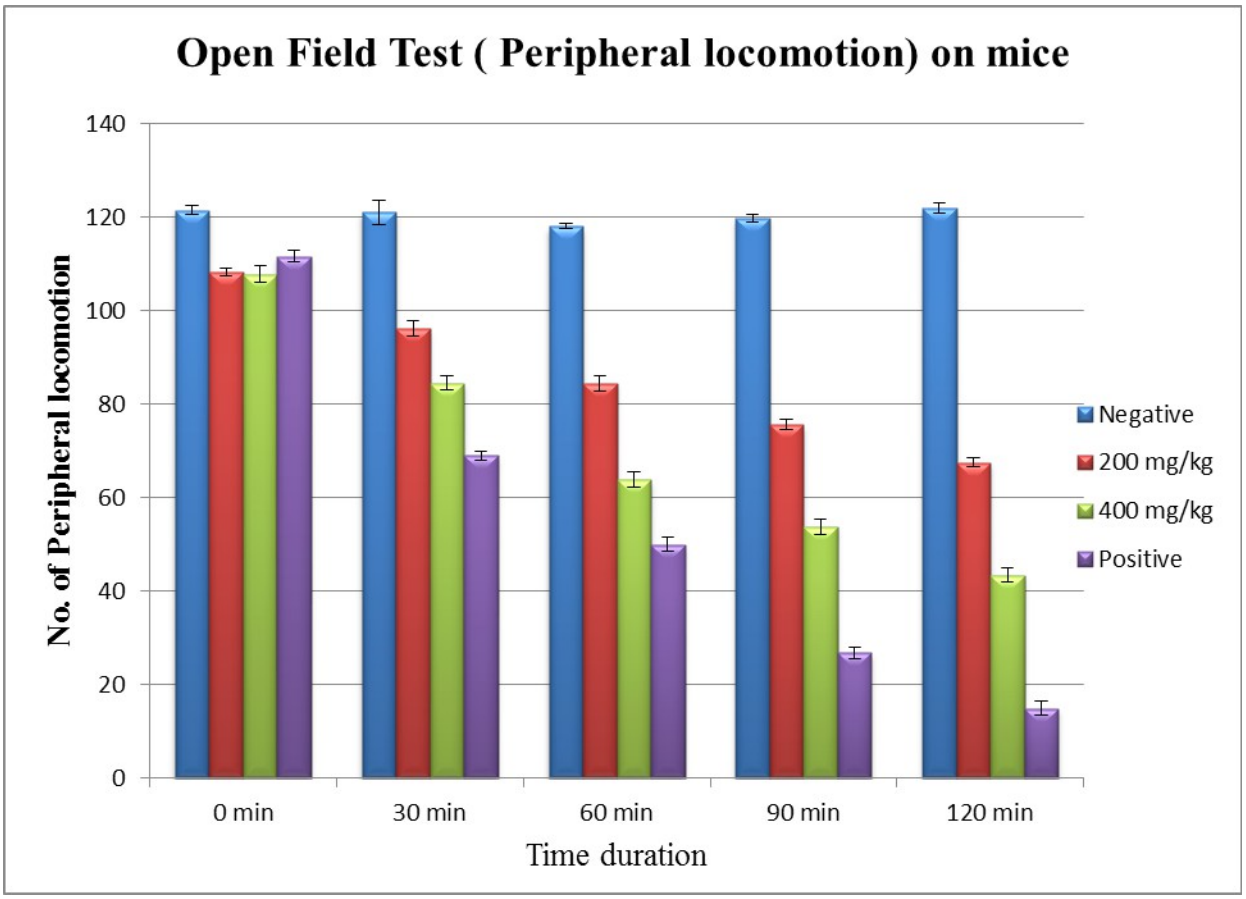
- ✓ The observed total number of central locomotion count is followed with a mean value of at 0 min 19.16±1.01***, at 30 min 9.83±0.79***, at 60 min 4.16±0.47***, at 90 min 3.00±0.51*** and at 120 min 2.0±0.25*** (Mean ±SEM) during 2 minutes observation.
- ✓ The observed total number of leaning count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

Table–6: CNS Activity of plant extract of *Mikania cordata* by Open Field Test (Peripheral Locomotion) in Mice.

Groups	Dose	No. of Peripheral Locomotion				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	121.50±1.05***	121±2.62***	118±0.56***	119.83±0.79***	121.83±1.10***
Crude extract of *Mikania cordata*	200mg/kg	108.33±0.88***	96.16±1.62***	84.50±1.64***	75.67±1.14***	67.50±0.99***
Crude extract of *Mikania cordata*	400mg/kg	107.83±1.81***	84.50±1.47***	63.83±1.62***	53.83±1.70***	43.50±1.56***
Positive control, Diazepam	1mg/kg	111.67±1.33***	69.0±1.06***	50.0±1.54***	26.83±1.19***	15.0±1.41***

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

Figure-24: Graphical Presentation of CNS Activity of plant extract of *Mikania cordata* by Open Field Test (Peripheral Locomotion) in Mice.

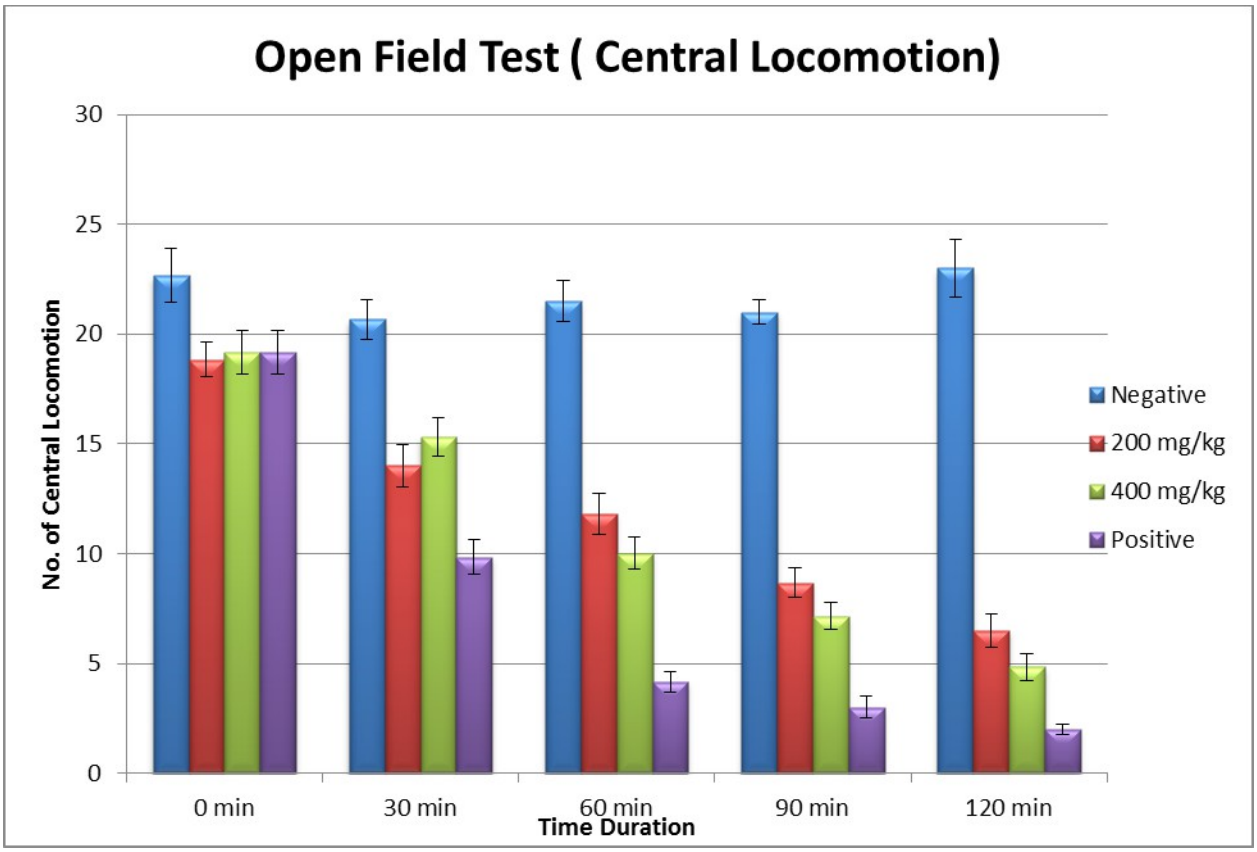


Table–7: CNS Activity of plant extract of *Mikania cordata* by Open Field Test (Central Locomotion) in Mice.

Groups	Dose	No. of Central Locomotion				
		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	22.67±1.22***	20.67±0.91***	21.50±0.92***	21.0±0.57***	23.0±1.31***
Crude extract of *Mikania cordata*	200mg/kg	18.83±0.79***	14.0±0.96***	11.83±0.94***	8.67±0.67***	6.50±0.76***
Crude extract of *Mikania cordata*	400mg/kg	19.16±1.01***	15.33±0.88***	10.0±0.73***	7.16±0.60***	4.83±0.60***
Positive control, Diazepam	1mg/kg	19.16±1.01***	9.83±0.79***	4.16±0.47***	3.00±0.51***	2.0±0.25***

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

Figure-25: Graphical Presentation of CNS Activity of plant extract of *Mikania cordata* by Open Field Test (Central Locomotion) in Mice.



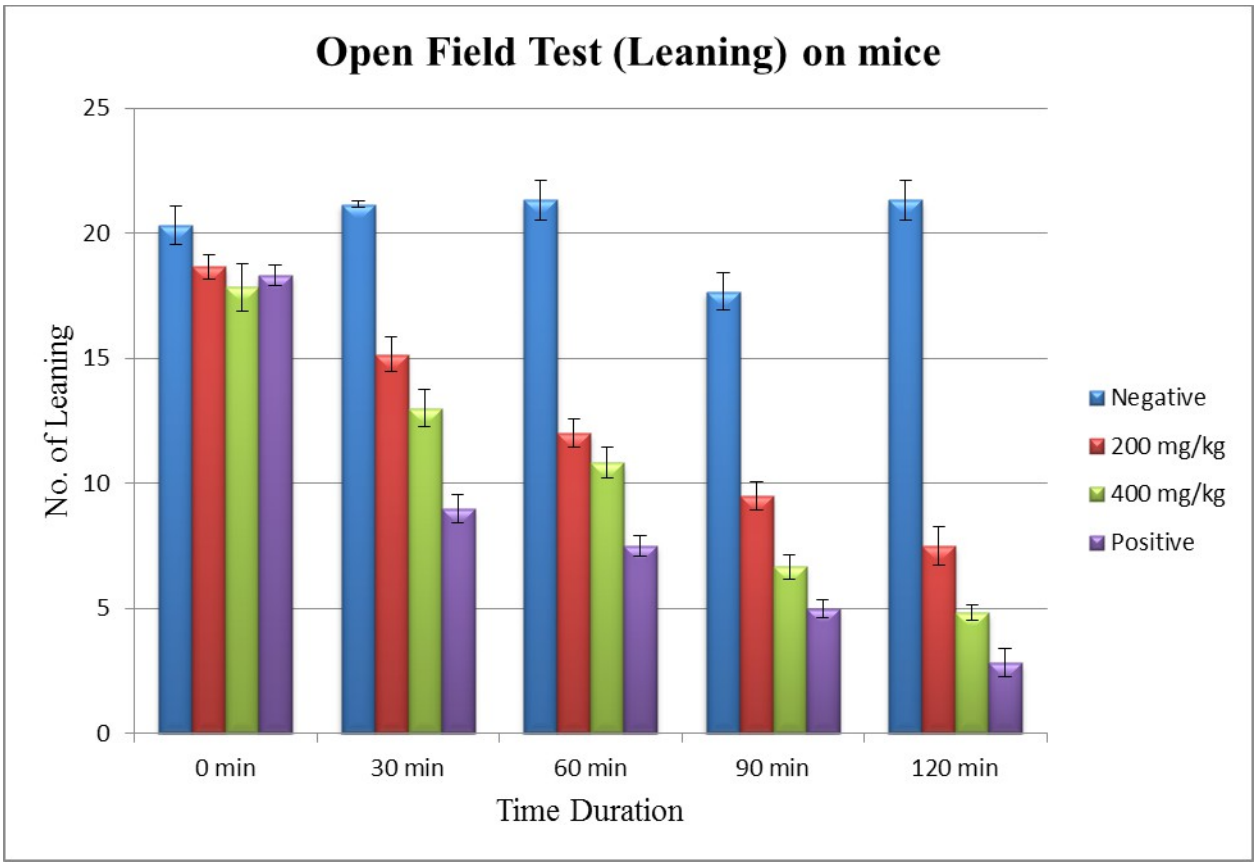
Table–8: CNS Activity of plant extract of *Mikania cordata* by Open Field Test (Leaning) in Mice.

Groups	Dose	No. of Leaning

		0 min	30 min	60 min	90 min	120 min
Negative control 5% CMC	10ml/kg	20.33±0.76***	21.16±0.13***	21.33±0.80***	17.67±0.76***	21.33±0.80***
Crude extract of *Mikania cordata*	200mg/kg	18.67±0.49***	15.16±0.70***	12.0±0.57***	9.50±0.56***	7.5±0.76***
Crude extract of *Mikania cordata*	400mg/kg	17.83±0.94***	13.0±0.73***	10.83±0.60***	6.67±0.49***	4.83±0.30***
Positive control, Diazepam	1mg/kg	18.33±0.42***	9.0±0.57***	7.5±0.42***	5.0±0.36***	2.83±0.54***

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

Figure-26: Graphical Presentation of CNS Activity of plant extract of *Mikania cordata* by Open Field Test (Leaning) in Mice.



4.1.2. Hole Board Test:

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

Total Number of Head Poking and Head Dipping count

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

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

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

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

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

This group of mice receive the plant extract of 400 mg/kg orally. The observed total number of head poking is with a mean value of 54.33±0.88 (Mean ±SEM) and head dipping with mean value of (Mean ±SEM) during 5 minutes observation after 30 min of administration.

Positive Control Group (Indomethacin, 10mg/kg)

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

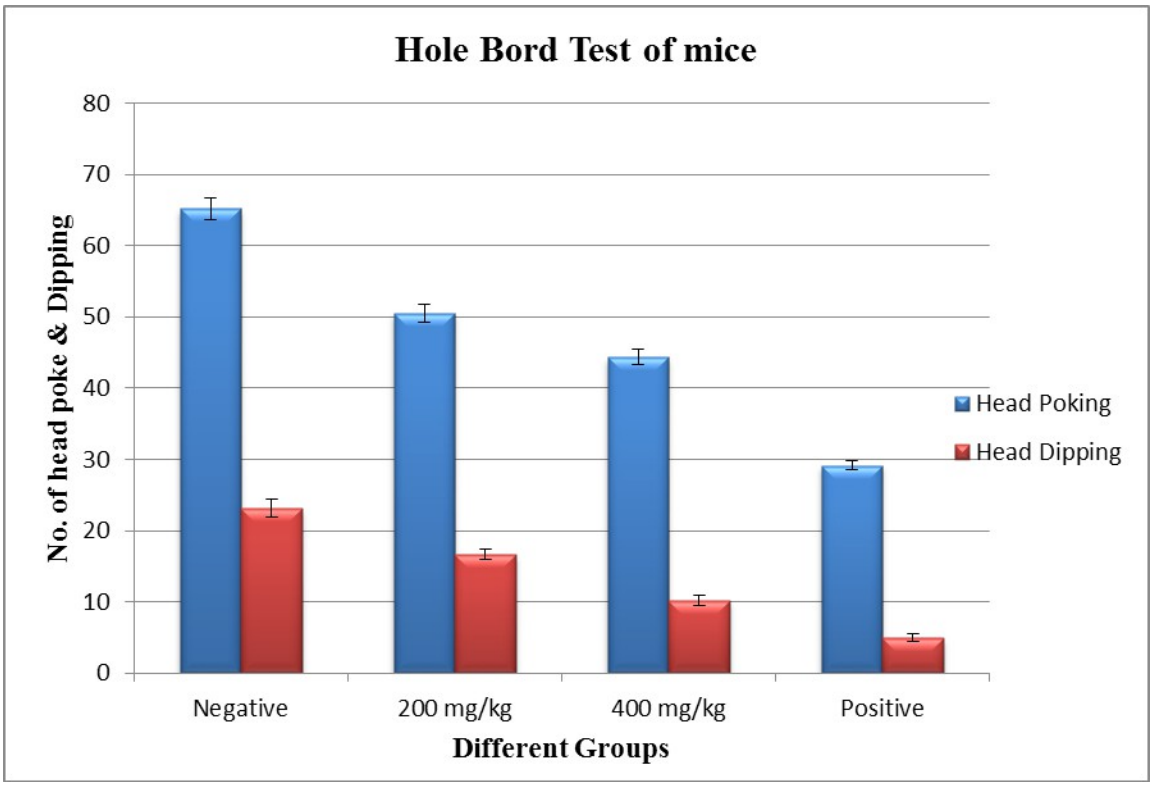
Table–9: CNS Activity of plant extract of *Mikania cordata* by Hole Board Test in Mice.

Groups	Treatment	Dose	No. of Head Poking	No. of Head Dipping
Negative control	5% CMC	10ml/kg	65.16±1.55***	23.16±1.19***

Group-1	Crude extract of *Mikania cordata*	200mg/kg	50.50±1.20***	16.67±0.67***
Group-2	Crude extract of *Mikania cordata*	400mg/kg	44.33±1.11***	10.16±0.70***
Positive control	Diazepam	1mg/kg	29.16±0.60***	5.0±0.57***

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

Figure–27: Graphical Presentation of CNS Activity of plant extract of *Mikania cordata* by Hole Board Test in Mice.



4.2. Analgesic Activity Test of Methanolic Extract of *Mikania cordata*

4.2.1. Acetic Acid Induced Writhing Test on Mice:

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

Total Number of writhing count and Percent Inhibition of Writhing:

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

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

Test Group-1 (Plant Extract, 800mg/kg)

This test group of mice receive the plant extract of 800 mg/kg orally. The total writhing count is followed with a mean value of 32.16±1.19 (Mean ±SEM) during 10 minutes observation. The observed P value is 0.000. The 800 mg/kg dose shows a highly significantly decrease in the total number of writhing. There is also significantly decreased the total number of writhing count found for the Indomethacin. The percent inhibition of writhing by this group that is pretreated with *Mikania cordata* (at a dose 800mg/kg as per body weight of mice) is 40.81% when it is compared to the negative control .

Test Group-2 (Plant Extract, 1000mg/kg)

This group of mice receive the plant extract of 1000 mg/kg orally. The total number of writhing count is followed with a mean value of 25.16± 0.94 (Mean ±SEM) during 10 minutes observation. The observed P value is 0.000. The 1000 mg/kg dose shows a highly significantly decrease in the total number of writhing count.. There are also significantly decrease in the total number of writhing count that is found for the Indomethacin .The percent inhibition of writhing by this group that is pretreated with *Mikania cordata* (at a dose 1000mg/kg as per body weight of mice) is 53.70%when it is compared to the negative control .

Test Group-3 (N-hexane Fraction of Plant Extract, 500mg/kg)

In this case of the test group of the mice receive the N-hexane fraction plant extract of 500 mg/kg orally. The total number of writhing count is followed with a mean value of 10.50±1.38 (Mean ±SEM) during 10 minutes observation. The observed P value is 0.000. The 500 mg/kg dose of N-hexane fraction of plant extract shows a highly significantly decrease in the total number of writhing count. There are also significantly decrease in the total number of writhing count that is found for the Indomethacin. The percent inhibition of writhing by this group that is pretreated with *Mikania cordata* (at a dose 400mg/kg as per body weight of mice) is 80.67%when it is compared to the negative control.

Test Group-4 (Dichloromethane Fraction of Plant Extract, 500mg/kg)

In this case of the test group of the mice receive the Dichloromethane fraction plant extract of 500 mg/kg orally. The total number of writhing count is followed with a mean value of 10.50±1.38 (Mean ±SEM) during 10 minutes observation. The observed P value is 0.000. The 500 mg/kg dose of Dichloromethane fraction of plant extract shows a highly significantly decrease in the total number of writhing count. There are also significantly decrease in the total number of writhing count that is found for the Indomethacin. The percent inhibition of writhing by this group that is pretreated with *Mikania cordata* (at a dose 500mg/kg as per body weight of mice) is 80.67%when it is compared to the negative control.

Positive Control Group (Indomethacin, 10mg/kg)

This group of mice receives the standard drug Indomethacin of 10mg/kg orally. The observed total number of writhing count is followed with a mean value of 18.67±1.02 (Mean ±SEM) during 10 minutes observation. The observed P value is 0.000. The positive control shows a highly significantly decrease in the total number of writhing count. The percent inhibition of writhing by this group that is pretreated with *Mikania cordata* (at a dose 10mg/kg as per body weight of mice) is 65.63%when compared to the negative control.

Table–10: Analgesic Activity of plant extract of *Mikania cordata* by Acetic Acid Induced Writhing test in Mice.

Groups	Treatment	Dose	No. of writhing	Percent inhibition
Negative control	5% CMC	10ml/kg	67.83±1.64***	-
Group-1	Crude extract of	800mg/kg	32.16±1.19***	52.58%

	Mikania cordata			
Group-2	Crude extract of *Mikania cordata*	1200mg/kg	25.16± 0.94***	62.90%
Group-3	N-hexane Fraction of *Mikania Cordata*	500mg/kg	49.50±0.76***	27.02%
Group-4	DCM Fraction of *Mikania Cordata*	500mg/kg	10.50±1.38***	84.52%
Positive control	Indomethacin	10mg/kg	18.67±1.02***	72.47%

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

Figure-28: Graphical Presentation of Analgesic Activity of plant extract of *Mikania cordata* by Acetic Acid Induced Writhing test in Mice.

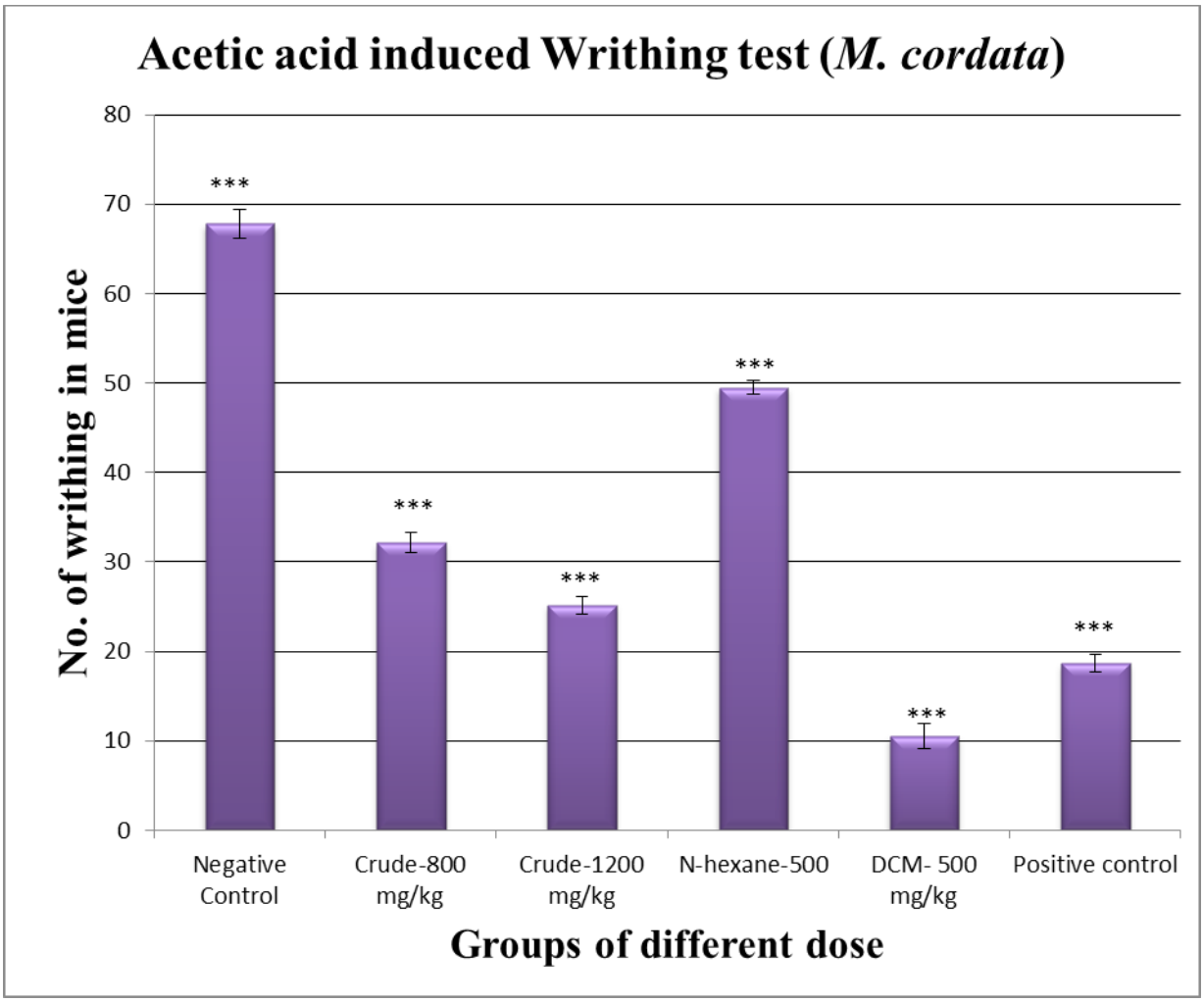
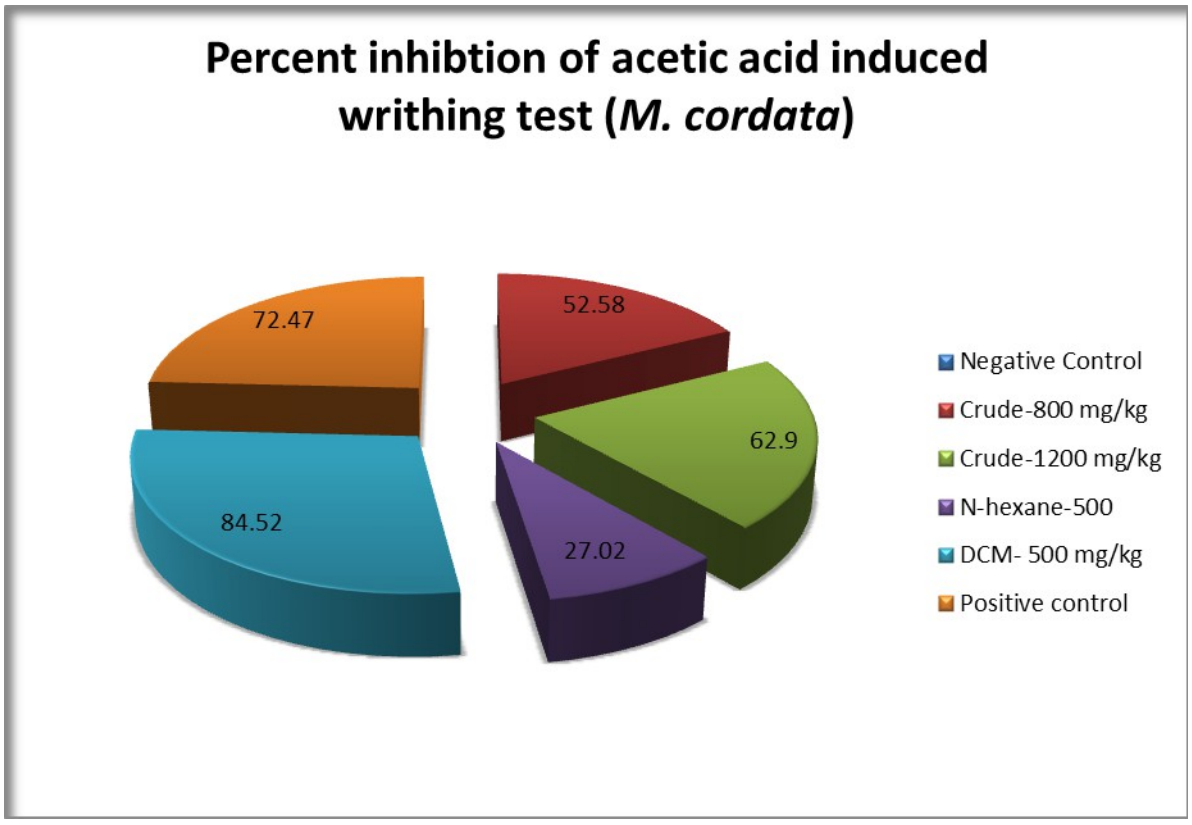


Figure-29: Percent inhibition of Analgesic Activity of plant extract of *Mikania cordata* by Acetic Acid Induced Writhing test in Mice.



4.2.2. Formalin Induced Hind Paw Licking in Mice:

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

Formalin Induced Licking Time of Swiss Albino Mice Counted in Two Phases & Percent Inhibition:

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

This group of animals only receive vehicle (5% CMC) 10ml/kg orally. The observed total paw licking times count for 1st phase (0-5th minutes after formalin injection) is considered as an anti-nociceptive phase and 2nd phase (15th -30th minutes after formalin injection) is considered as an anti-inflammatory phase are followed with a mean value of 184.17±1.30 and 185.83±0.95 (Mean ±SEM).

Test Group-1 (Plant Extract, 800mg/kg)

This test group of mice receive the plant extract of 800 mg/kg orally.

1st phase: The observed paw licking times count for 1st phase (0-5th minutes after formalin injection) are followed with a mean value of 152.67±1.92 (Mean ±SEM) during first five minutes observation. The observed P values are 0.000.

2nd phase: The observed paw licking times count for 2nd phase (15th -30th minutes after formalin injection) are followed with a mean value of 113.50±1.33 (Mean ±SEM) during last fifteen minutes observation. The observed P values are 0.000.

The 800 mg/kg dose shows a highly significantly decrease in the paw licking times in both phases. There are also significantly decrease in the paw licking times found for the Aspirin.

The percent inhibition of licking times in both phases at a dose 800mg/kg as per body weight of mice are 17.09% for 1st phase and 38.92% for 2nd phase when compared to the negative control.

Test Group-2 (Plant Extract, 1200mg/kg)

This test group of mice receive the plant extract of 1200 mg/kg orally.

1st phase: The observed paw licking times count for 1st phase (0-5th minutes after formalin injection) are followed with a mean value of 129.83±1.22 (Mean ±SEM) during first five minutes observation. The observed P values are 0.000.

2nd phase: The observed paw licking times count for 2nd phase (15th -30th minutes after formalin injection) are followed with a mean value of 83.33±0.88 (Mean ±SEM) during last fifteen minutes observation. The observed P values are 0.000.

The 1200 mg/kg dose shows a highly significantly decrease in the paw licking times in both phases. There are also significantly decrease in the paw licking times found for the Aspirin.

The percent inhibition of licking times in both phases at a dose 400mg/kg as per body weight of mice are 29.50% for 1st phase and 55.15%% for 2nd phase when compared to the negative control

Test Group-3 (N-Hexane fraction, 500mg/kg)

This test group of mice receive the N-Hexane fraction of plant extract, 500mg/kg orally.

1st phase: The observed paw licking times count for 1st phase (0-5th minutes after formalin injection) are followed with a mean value of 175.00±1.00 (Mean ±SEM) during first five minutes observation. The observed P values are 0.000.

2nd phase: The observed paw licking times count for 2nd phase (15th -30th minutes after formalin injection) are followed with a mean value of 175.83±0.94 (Mean ±SEM) during last fifteen minutes observation. The observed P values are 0.000.

The 500 mg/kg dose of N-Hexane fraction shows not any significantly decrease in the paw licking times in both phases.

The percent inhibition of licking times in both phases at a dose 500 mg/kg dose of N-Hexane fraction as per body weight of mice are 4.97% for 1st phase and 5.38%% for 2nd phase when compared to the negative control.

Test Group-4 (DCM fraction, 500mg/kg)

This test group of mice receive the DCM fraction of plant extract, 500mg/kg orally.

1st phase: The observed paw licking times count for 1st phase (0-5th minutes after formalin injection) are followed with a mean value of 82.67±0.76 (Mean ±SEM) during first five minutes observation. The observed P values are 0.000.

2nd phase: The observed paw licking times count for 2nd phase (15th -30th minutes after formalin injection) are followed with a mean value of 63.50±0.76 (Mean ±SEM) during last fifteen minutes observation. The observed P values are 0.000.

The 500 mg/kg dose of N-Hexane fraction shows a highly significantly decrease in the paw licking times in both phases.

The percent inhibition of licking times in both phases at a dose 500 mg/kg dose of N-Hexane fraction as per body weight of mice are 55.10% for 1st phase and 65.82% for 2nd phase when compared to the negative control.

Positive Control (Aspirin, 100mg/kg)

This test group of mice receive the standard drug, Aspirin of 100mg/kg orally.

1st phase: The observed paw licking times count for 1st phase (0-5th minutes after formalin injection) are followed with a mean value of 92.67±0.77 (Mean ±SEM) during first five minutes observation. The observed P values are 0.000.

2nd phase: The observed paw licking times count for 2nd phase (15th -30th minutes after formalin injection) are followed with a mean value of 73.50±0.77 (Mean ±SEM) during last fifteen minutes observation. The observed P values are 0.000.

The positive control shows a highly significantly decrease in the time of paw licking count when compared to the negative control.

The percent inhibition of licking times in both phases by this group pretreated with Aspirin (at a dose 100mg/kg as per body weight of mice) are 49.69% for 1st phase and 60.45% for 2nd phase when compared to the negative control.

Table –11: Analgesic Activity of Plant Extract of *Mikania cordata* in Formalin Induced Hind Paw Licking in Mice.

Groups	Treatment	Dose	Mean licking time (in Sec) 1st phase (0-5th min)	Percent inhibition 1st phase (0-5th min)	Mean licking time (in sec) 2nd phase (15-30th min)	Percent inhibition 2nd phase (15-30th min)
Negative control	5% CMC	10ml/kg	184.16±1.30***		185.83±0.94***	
Group-1	Crude extract of *Mikania cordata*	800mg/kg	152.67±1.92***	17.09%	113.50±1.33***	38.92%
Group-2	Crude extract of *Mikania cordata*	1200mg/kg	129.83±1.22***	29.50%	83.33±0.88***	55.15%
Group-4	N-hexane fraction of *Mikania cordata*	500mg/kg	175.00±1.00***	4.97%	175.83±0.94***	5.38%
Group-5	DCM fraction of *Mikania cordata*	500mg/kg	82.67±0.76***	55.10%	63.50±0.76***	65.82%
Positive control	Aspirin	100mg/kg	92.66±0.76***	49.68%	73.50±0.76***	60.44%

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

Figure –30: Graphical Presentation of Analgesic Activity of Plant Extract of *Mikania cordata* in Formalin Induced Hind Paw Licking in Mice (1st phase).

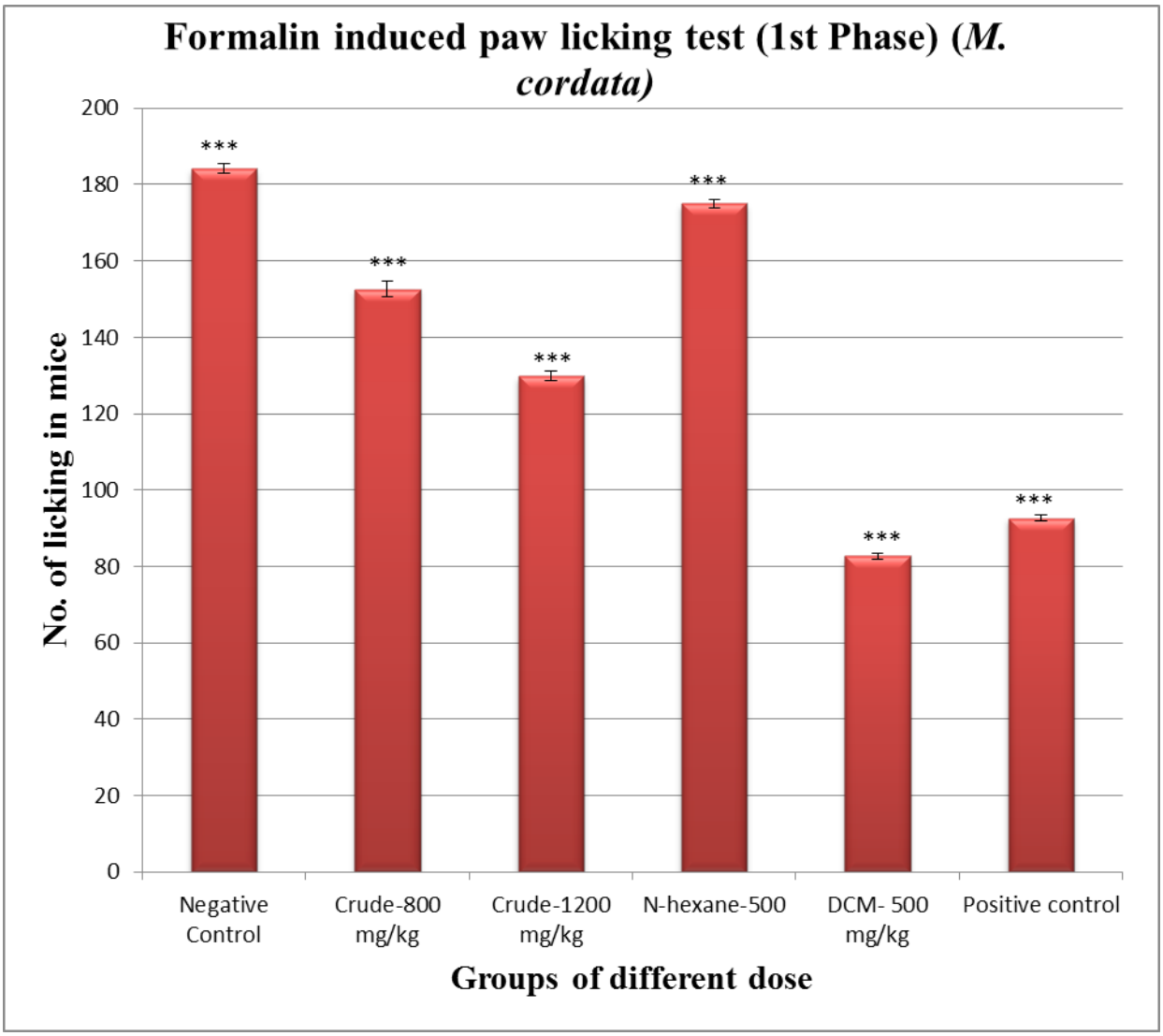


Figure –31: Percent Inhibition of Analgesic Activity of Plant Extract of *Mikania cordata* in Formalin Induced Hind Paw Licking in Mice (1st phase).

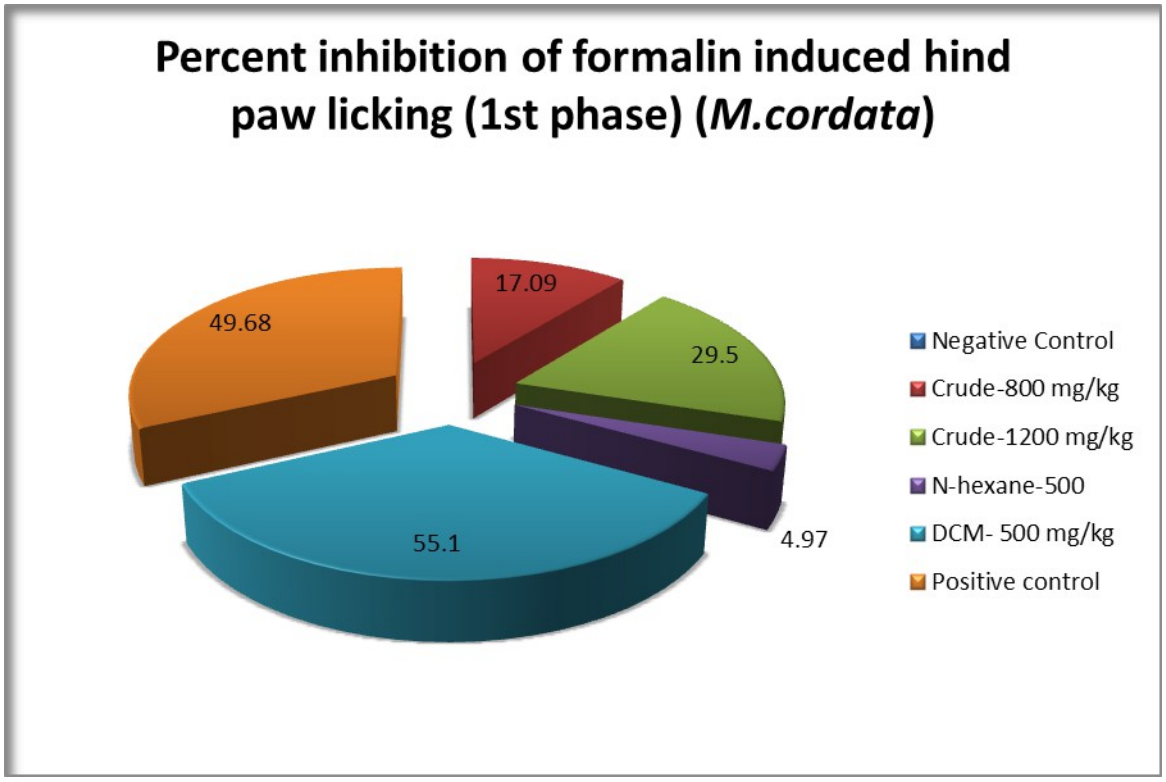


Figure –32: Graphical Presentation of Analgesic Activity of Plant Extract of *Mikania cordata* in Formalin Induced Hind Paw Licking in Mice (2nd phase).

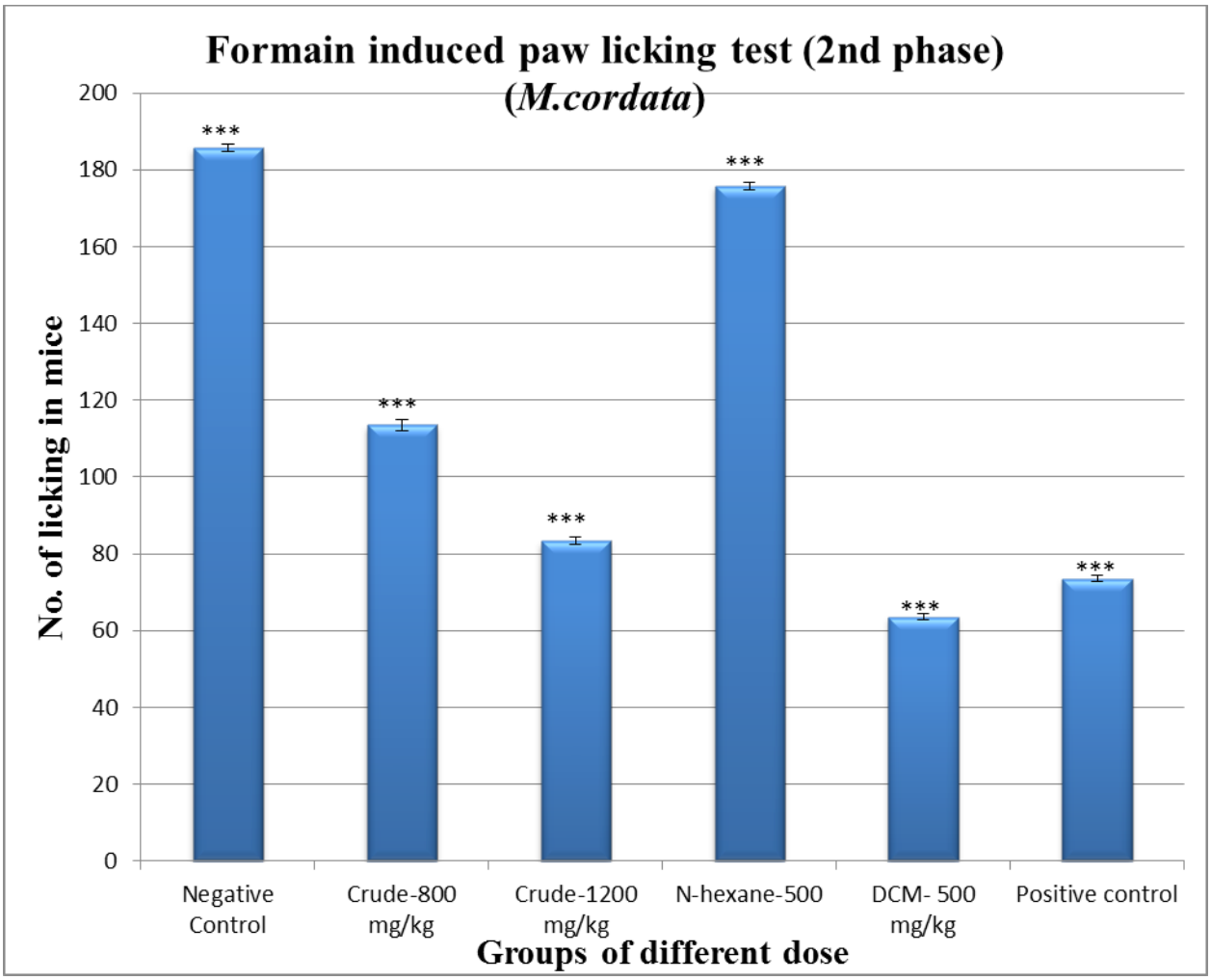
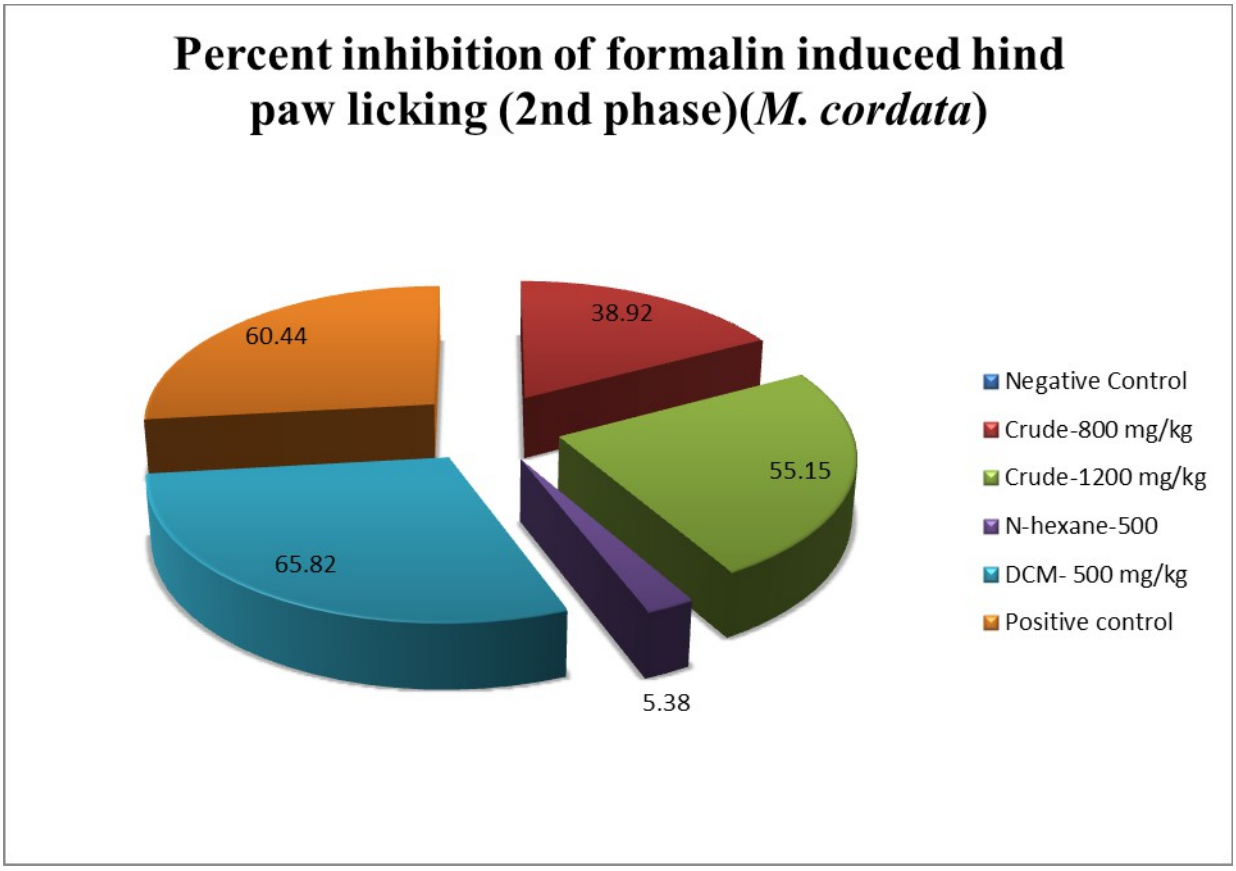


Figure –33: Percent Inhibition of Analgesic Activity of Plant Extract of *Mikania cordata* in Formalin Induced Hind Paw Licking in Mice (1st phase).



4.3. Analgesic Activity Test of Methanolic Extract of *Spilanthes acmella*

4.3.1. Acetic Acid Induced Writhing Test on Mice:

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

Total Number of writhing count and Percent Inhibition of Writhing:

Negative Control Group (1% Tween 80 vehicle, 10 ml/kg)

This group of animals only received vehicle (1% Tween 80 vehicle) 10 ml/kg orally. The observed total writhing count is followed with a mean value of 62.84 ± 1.01 (Mean ±SEM) during 10 minutes observation.

Test Group-1 (n-hexane fraction, 200mg/kg)

This test group of mice receive the n-hexane fraction of 200 mg/kg orally. The total writhing count is followed with a mean value of 45.17±1.17 (Mean ±SEM) during 10 minutes observation. The observed P value is 0.000. The 200 mg/kg dose shows a highly significantly decrease in the total number of writhing. There is also significantly decreased the total number of writhing count found for the Indomethacin. The percent inhibition of writhing by this group that is pretreated with *Spilanthes acmella* (at a dose 800mg/kg as per body weight of mice) is 54.65% when it is compared to the negative control .

Test Group-2 (Chloroform fraction, 200mg/kg)

This group of mice receive the Chloroform fraction of 200 mg/kg orally. The total number of writhing count is followed with a mean value of 11.67 ± 0.67 (Mean ±SEM) during 10 minutes observation. The observed P value is 0.000. The 200 mg/kg dose shows a highly significantly decrease in the total number of writhing count. There are also significantly decrease in the total number of writhing count that is found for the Indomethacin .The percent inhibition of writhing by this group that is pretreated with *Spilanthes acmella* (at a dose 200mg/kg as per body weight of mice) is 81.42%when it is compared to the negative control .

Test Group-3 (Ethyl acetate fraction, 200mg/kg)

In this case of the test group of the mice receive the Ethyl acetate fraction of 200 mg/kg orally. The total number of writhing count is followed with a mean value of 35.67 ± 0.89 (Mean ±SEM) during 10 minutes observation. The observed P value is 0.000. The 300 mg/kg dose of Ethyl acetate fraction shows not only significantly decrease in the total number of writhing count.

There are also significantly decrease in the total number of writhing count that is found for the Indomethacin. The percent inhibition of writhing by this group that is pretreated with *Spilanthes acmella* (at a dose 200mg/kg as per body weight of mice) is 43.24%when it is compared to the negative control.

Test Group-4 (Water Fraction, 200mg/kg)

In this case of the test group of the mice receive the Water fraction of 200 mg/kg orally. The total number of writhing count is followed with a mean value of 44.50 ± 1.34 (Mean ±SEM) during 10 minutes observation. The observed P value is 0.000. The 200 mg/kg dose of water fraction of plant extract shows not any significantly decrease in the total number of writhing count. There are also significantly decrease in the total number of writhing count that is found for the Indomethacin. The percent inhibition of writhing by this group that is pretreated with *Spilanthes acmella* (at a dose 200mg/kg as per body weight of mice) is 29.18%when it is compared to the negative control.

Positive Control Group (Indomethacin, 10mg/kg)

This group of mice receives the standard drug Indomethacin of 10mg/kg orally. The observed total number of writhing count is followed with a mean value of 23.67 ± 0.89 (Mean ±SEM) during 10 minutes observation. The observed P value is 0.000. The positive control shows a highly significantly decrease in the total number of writhing count. The percent inhibition of writhing by this group that is pretreated with *Spilanthes acmella*(at a dose 10mg/kg as per body weight of mice) is 62.34% when compared to the negative control.

Table –12: Analgesic Activity of plant extract of *Spilanthes acmella* by Acetic Acid Induced Writhing test in Mice.

Groups	Treatment	Dose	No. of writhing	Percent inhibition
Group-1	Negative control	10ml/kg	62.84 ± 1.01	
Group-2	n-hexane fraction	200mg/kg	28.50 ± 0.77***	54.65%
Group-3	Chloroform Fraction	200mg/kg	11.67 ± 0.67***	81.42%
Group-4	Ethyl acetate fraction	200mg/kg	35.67 ± 0.89***	43.24%
Group-5	Water fraction	200mg/kg	44.50 ± 1.34***	29.18%
Group-6	Positive control	10mg/kg	23.67 ± 0.89***	62.34%

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

Figure-34: Graphical Presentation of Analgesic Activity of plant extract of *Spilanthes acmella* by Acetic Acid Induced Writhing test in Mice.

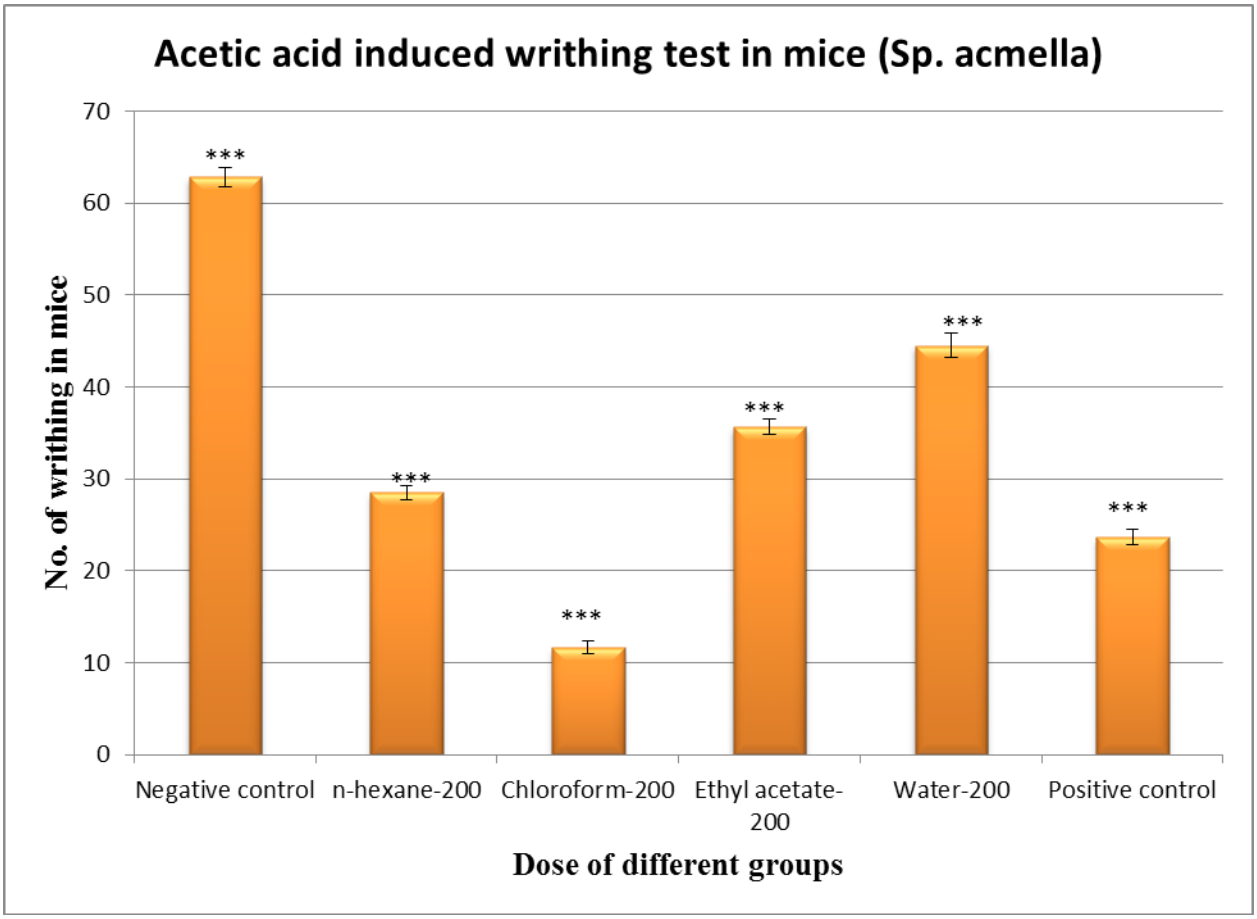
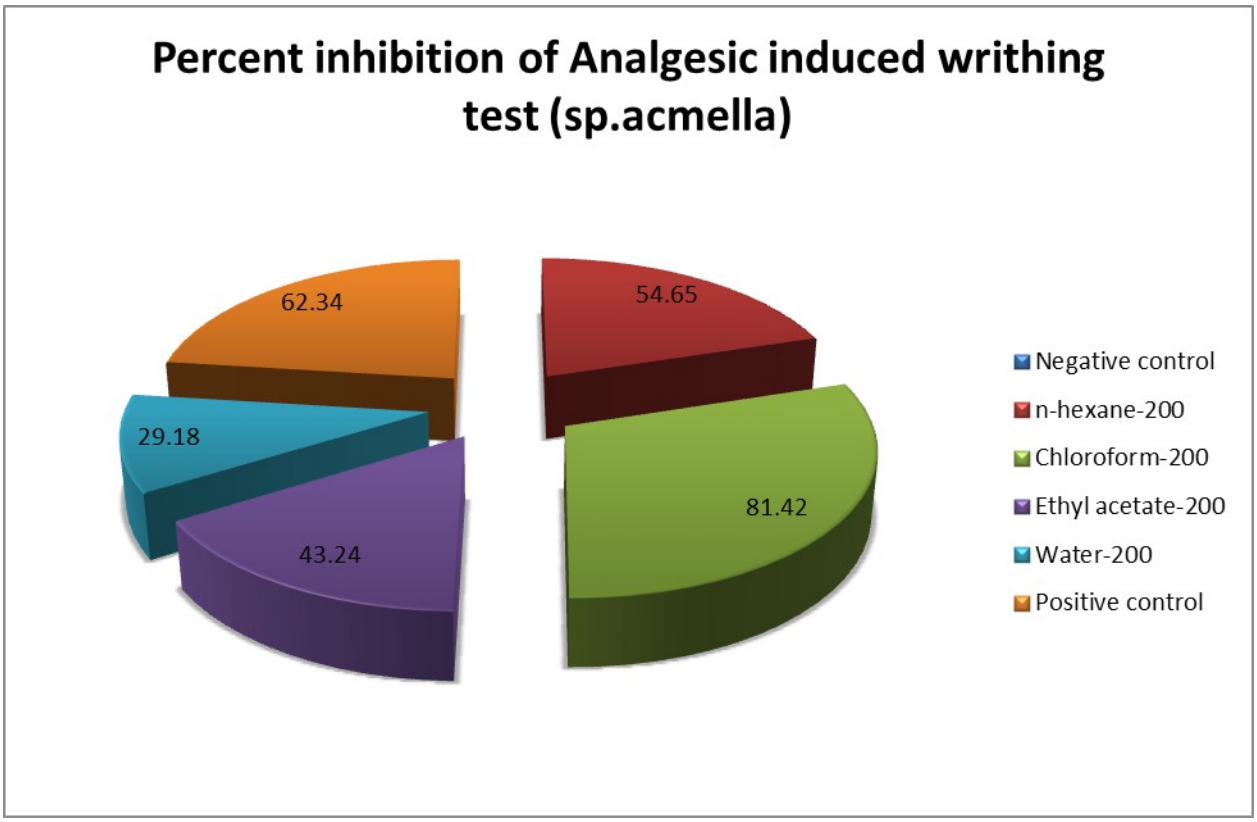


Figure-35: Analgesic Activity of plant extract of *Spilanthes acmella* by Acetic Acid Induced Writhing test in Mice.



4.3.2. Formalin Induced Hind Paw Licking in Mice:

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

Formalin Induced Licking Time of Swiss Albino Mice Counted in Two Phases & Percent Inhibition:

Negative Control Group (1% Tween 80 vehicle, 10ml/kg)

This group of animals only receive vehicle (1% Tween 80 vehicle) 10ml/kg orally. The observed total paw licking times count for 1st phase (0-5th minutes after formalin injection) is considered as an anti-nociceptive phase and 2nd phase (15th -30th minutes after formalin injection) is considered as an anti-inflammatory phase are followed with a mean value of 82.84±1.30and 75.84±0.95 (Mean ±SEM).

Test Group-1 (n-hexane fraction, 200mg/kg)

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

1st phase: The observed paw licking times count for 1st phase (0-5th minutes after formalin injection) are followed with a mean value of 75.67±1.20 (Mean ±SEM) during first five minutes observation. The observed P values are 0.000.

2nd phase: The observed paw licking times count for 2nd phase (15th -30th minutes after formalin injection) are followed with a mean value of 65.66±1.36 (Mean ±SEM) during last fifteen minutes observation. The observed P values are 0.000.

The 200 mg/kg dose shows a highly significantly decrease in the paw licking times in both phases. There are also significantly decrease in the paw licking times found for the Aspirin.

The percent inhibition of licking times in both phases at a dose 200mg/kg as per body weight of mice are 8.65%for 1st phase and 13.42% for 2nd phase when compared to the negative control .

Test Group-2 (Chloroform fraction, 200mg/kg)

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

1st phase: The observed paw licking times count for 1st phase (0-5th minutes after formalin injection) are followed with a mean value of 61.50±0.76 (Mean ±SEM) during first five minutes observation. The observed P values are 0.000.

2nd phase: The observed paw licking times count for 2nd phase (15th -30th minutes after formalin injection) are followed with a mean value of 57.00±0.74 (Mean ±SEM) during last fifteen minutes observation. The observed P values are 0.000.

The 200 mg/kg dose shows a highly significantly decrease in the paw licking times in both phases. There are also significantly decrease in the paw licking times found for the Aspirin.

The percent inhibition of licking times in both phases at a dose 400mg/kg as per body weight of mice are 25.76%for 1st phase and 24.84% for 2nd phase when compared to the negative control

Positive Control (Aspirin, 100mg/kg)

This test group of mice receive the standard drug, Aspirin of 100mg/kg orally.

1st phase: The observed paw licking times count for 1st phase (0-5th minutes after formalin injection) are followed with a mean value of 42.67±0.67 (Mean ±SEM) during first five minutes observation. The observed P values are 0.000.

2nd phase: The observed paw licking times count for 2nd phase (15th -30th minutes after formalin injection) are followed with a mean value of 36.67±0.67 (Mean ±SEM) during last fifteen minutes observation. The observed P values are 0.000.

The positive control shows a highly significantly decrease in the time of paw licking count when compared to the negative control.

The percent inhibition of licking times in both phases by this group pretreated with Aspirin (at a dose 100mg/kg as per body weight of mice) are 48.49%for 1st phase and 51.64%for 2nd phase when compared to the negative control.

Table –13: Analgesic Activity of plant extract of *Spilanthes acmella* in Formalin induced hind paw licking in mice.

Groups	Treatment	Dose	Mean(1st phase) (in Sec)	Percent inhibition	Mean(2nd phase) (in sec)	Percent inhibition
Group-1	1% Tween80	10ml/kg	82.84±1.30***		75.84±0.95***	
Group-2	n-hexane fraction (*Spilanthes acmella*)	200mg/kg	75.67±1.20***	8.65%	65.66±1.36***	13.42%
Group-3	Chloroform fraction (*Spilanthes acmella*)	200mg/kg	61.50±0.76***	25.76%	57.00±0.74***	24.84%
Group-4	Aspirin	100mg/kg	42.67±0.67***	48.49%	36.67±0.67***	51.64%

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

Figure-36: Graphical Presentation of Analgesic Activity of plant extract of *Spilanthes acmella* in Formalin induced hind paw licking in mice (1st phase).

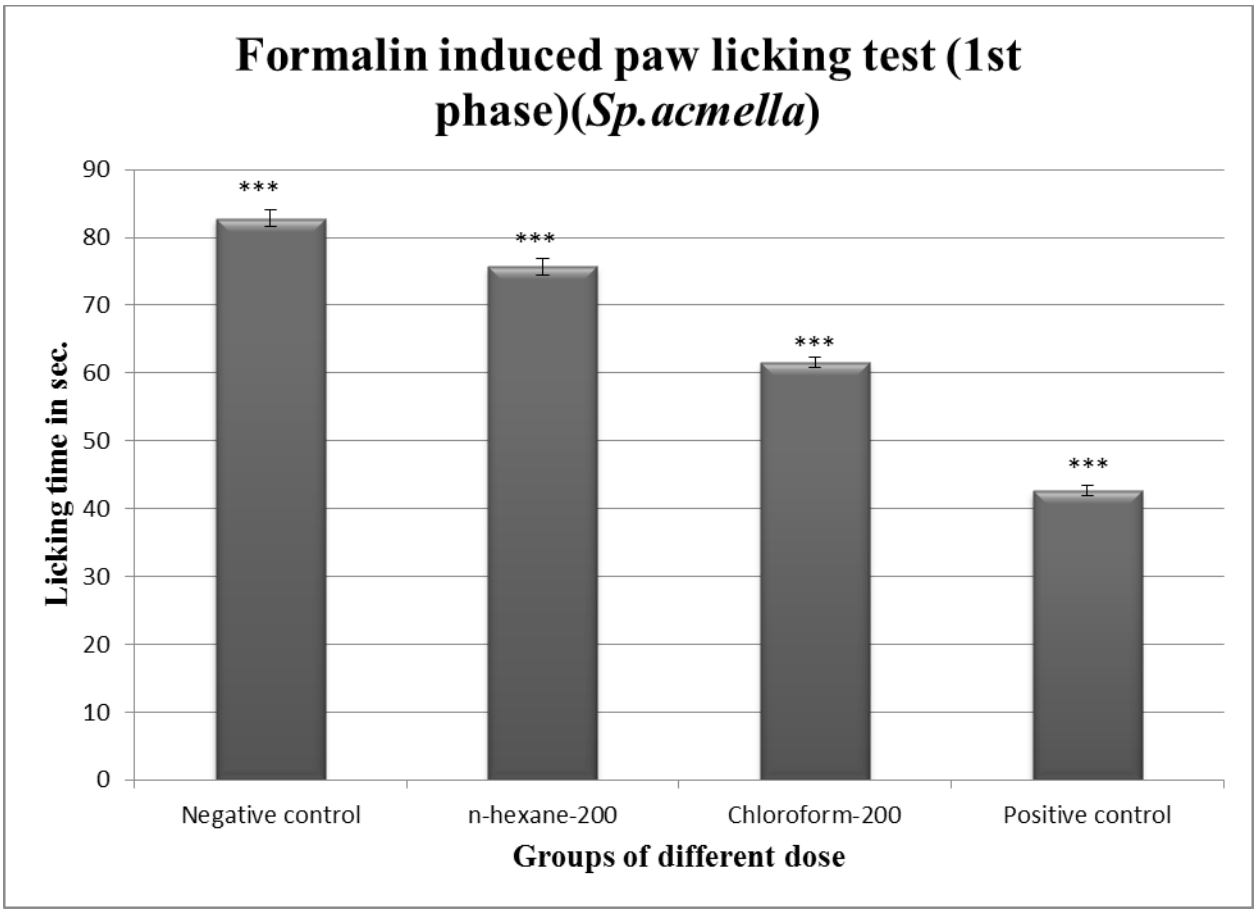


Figure- 37: Percent inhibition of Analgesic Activity of plant extract of *Spilanthes acmella* in Formalin induced hind paw licking in mice (1st phase).

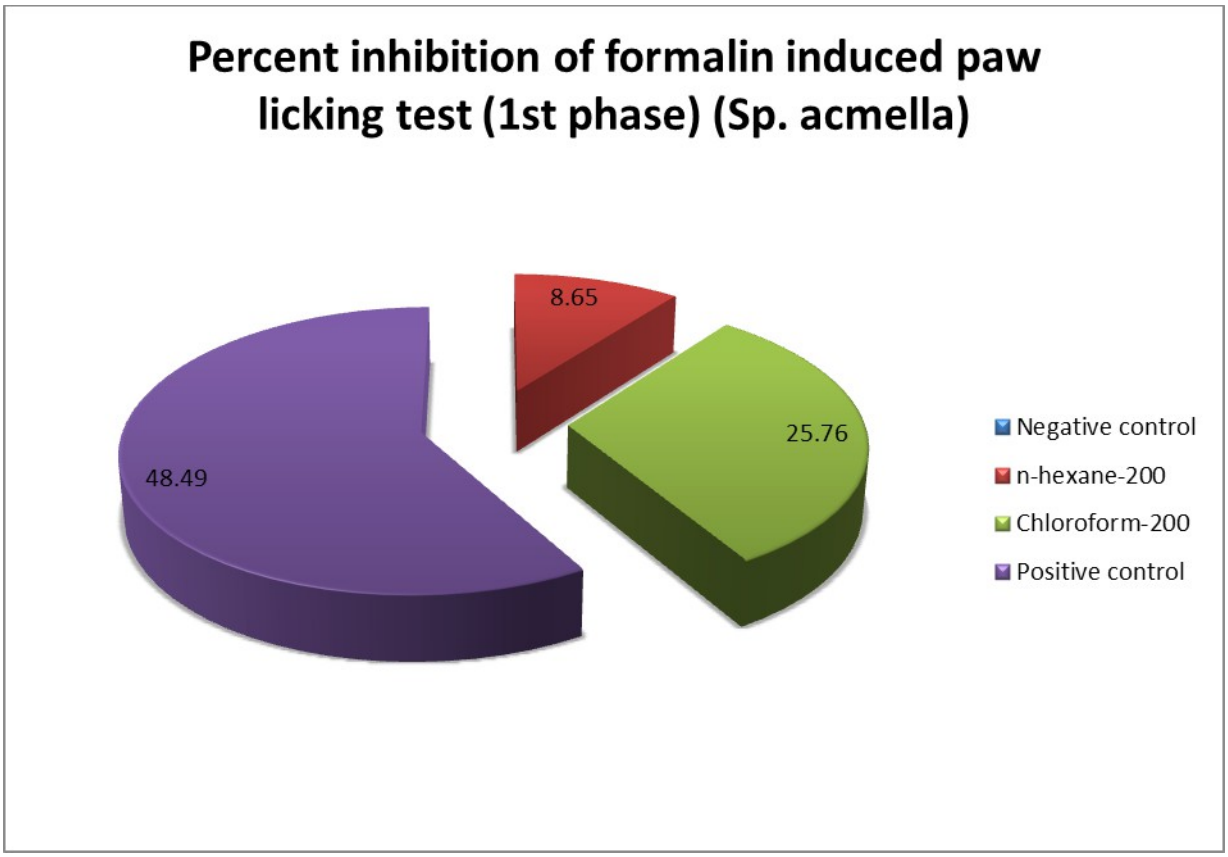


Figure- 38: Graphical Presentation of Analgesic Activity of plant extract of *Spilanthes acmella* in Formalin induced hind paw licking in mice (2nd phase).

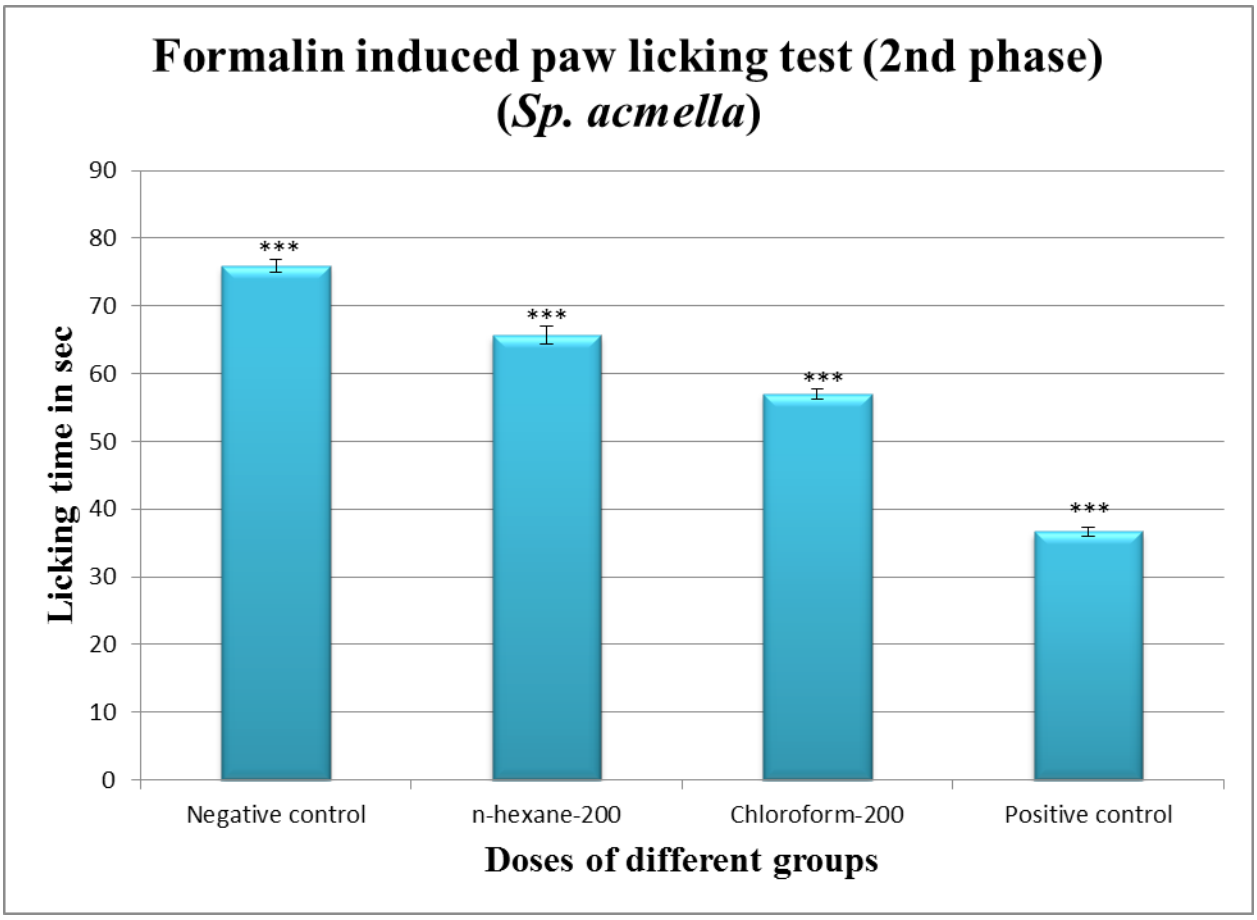
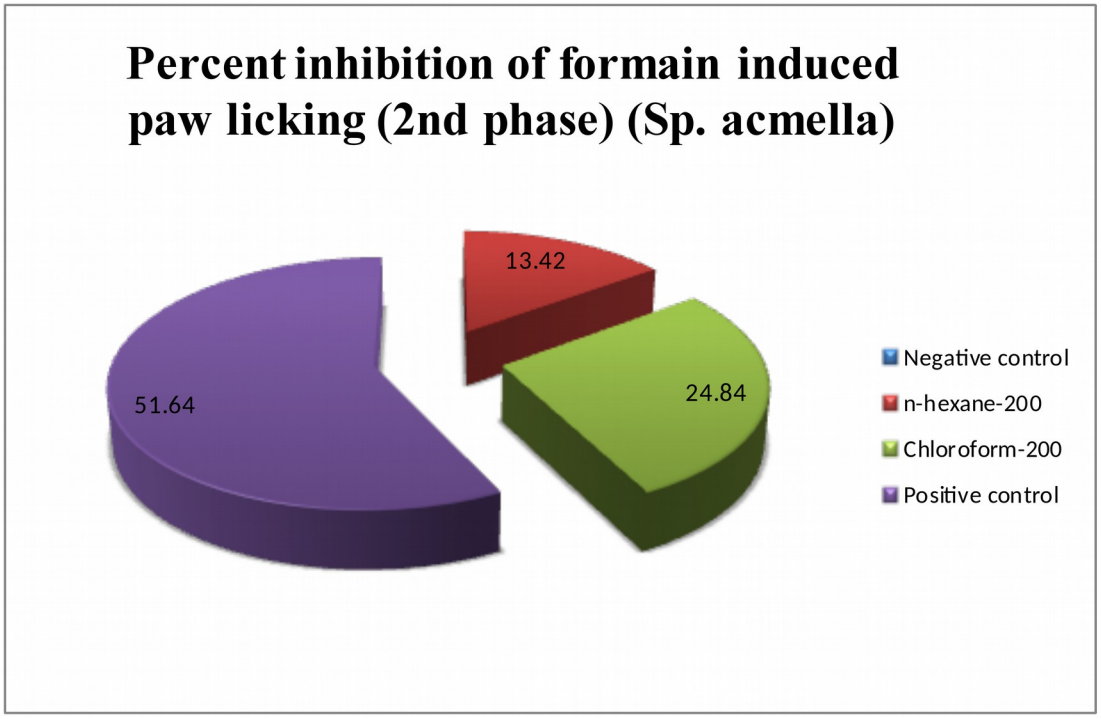


Figure- 39: Percent inhibition of Analgesic Activity of plant extract of *Spilanthes acmella* in Formalin induced hind paw licking in mice (2nd phase).



4.4. Acute and Sub Chronic Toxicity Test

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

4.4.2. Sub Chronic Toxicity Test:

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

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

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

Table-14: Effect of methanolic extract of *Mikania cordata* on body weight in mice

Treatment Groups	Gender	Initial body weight	Final body weight	No. of death
Normal Control (5% CMC)	Male	31.89±1.28***	40.84±1.43***	3
***Mikania cordata*-200mg/kg**	Male	28.38±0.58***	36.60±1.30***	3
***Mikania cordata*-400mg/kg**	Female	29.12±0.55***	33.11±1.28***	4
***Mikania cordata*-600mg/kg**	Female	28.70±0.97***	30.86±1.64***	4

Figure-40: Graphical Presentation of Effect of methanolic extract of *Mikania cordata* on body weight in mice

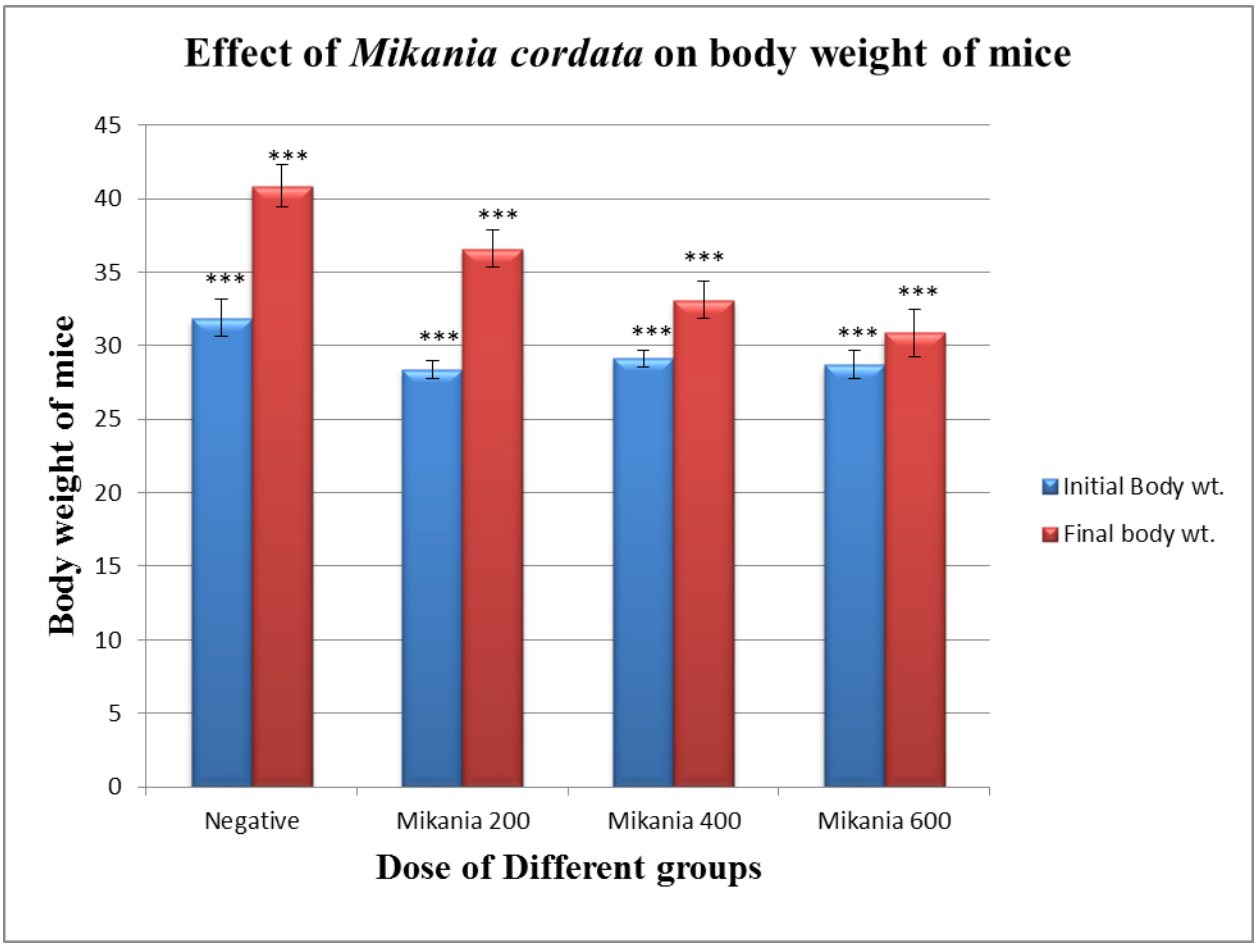


Table-15: Effect of *Mikania cordata* on the WBC count (White Blood Cell)

Treatment Group	Total WBC $10^3/mm^3$(n)	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil

Negative Control group(5% CMC)	4.07	13.9	82.2	8.25	19.65	6.96
***Mikania cordata* (200mg/kg)**	3.39	13.6	54.2	7.9	19.45	4.8
***Mikania cordata* (400mg/kg)**	2.60	12	48.15	4.7	12.96	8.23
***Mikania cordata* (600mg/kg)**	0.33	11.65	45	2.85	8	3.05

Figure-41: Effect of *Mikania cordata* on the WBC (White Blood Cell) count

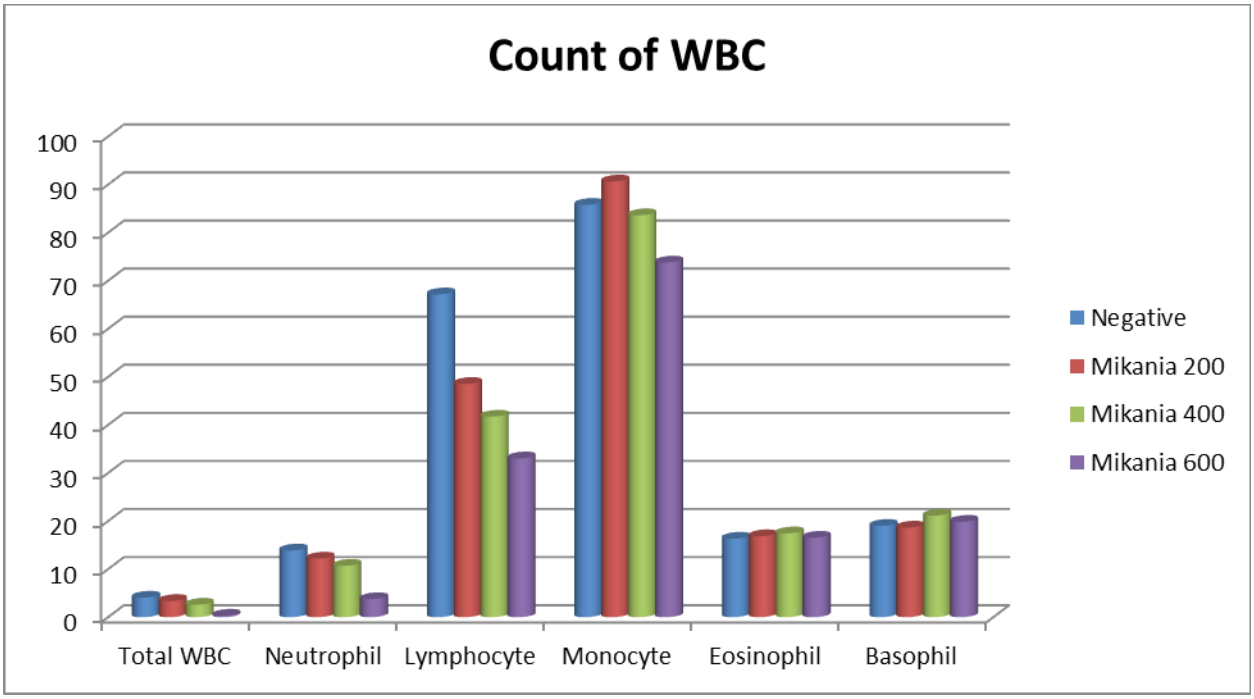


Table-16: Effect of *Mikania cordata* on the RBC (Red Blood Cell) count

Treatment Group	Total RBC10^6/ mm^3(n)	Haemoglobin	HCT	MCV	MCH	MCHC	RDW

Negative Control group(5% CMC)	8.08	13.9	67.17	85.8	16.35	19	19.37
***Mikania cordata* (200mg/kg)**	5.37	12.2	48.55	90.65	16.85	18.65	18.45
***Mikania cordata* (400mg/kg)**	5.05	10.7	41.73	83.63	17.45	21.15	18.26
***Mikania cordata* (600mg/kg)**	2.28	3.77	33.05	73.8	16.55	19.86	19.86

Figure-42: Effect of *Mikania cordata* on the count of RBC (Red Blood Cell)

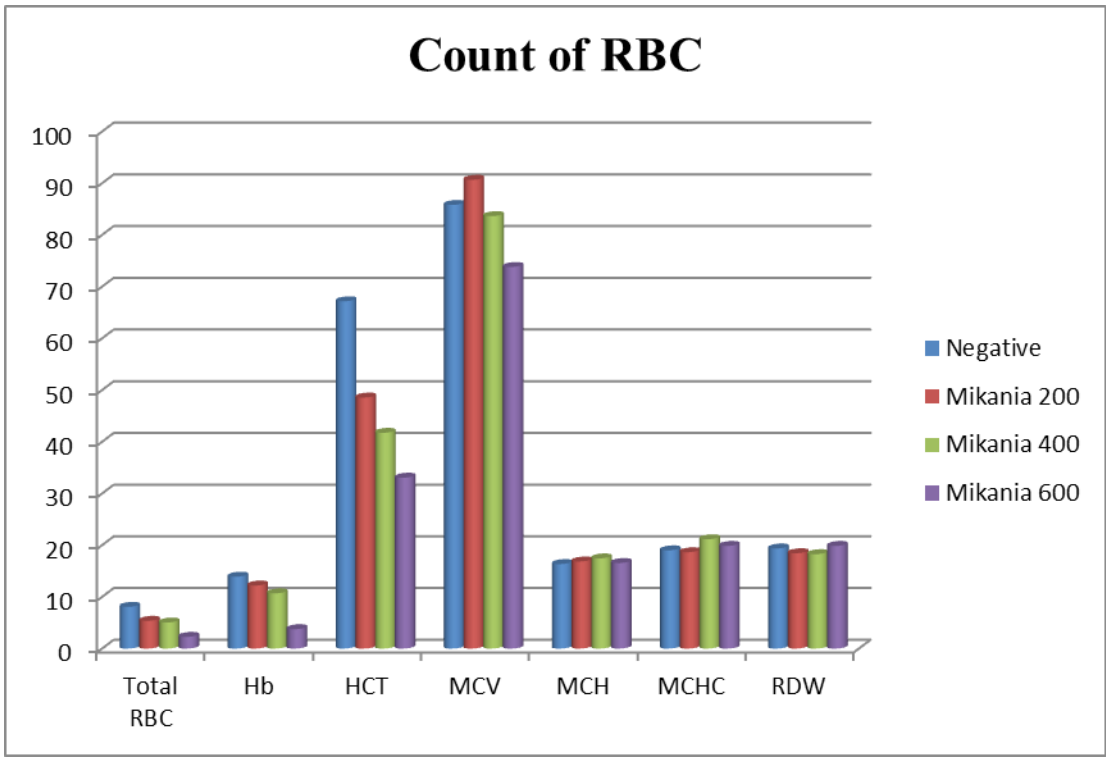


Table-17: Effect of *Mikania cordata* on Platelet count on the CBC (Count Blood Cell) Test

Treatment Group	Platelet $10^3/mm^3$(n)

Negative Control group(5% CMC solution)	1274
***Mikania cordata* (200mg/kg)**	1007
***Mikania cordata* (400mg/kg)**	860
***Mikania cordata* (600mg/kg)**	255

Figure-43: Effect of Mikania cordata on Platelet on the CBC (Count Blood Cell) Test

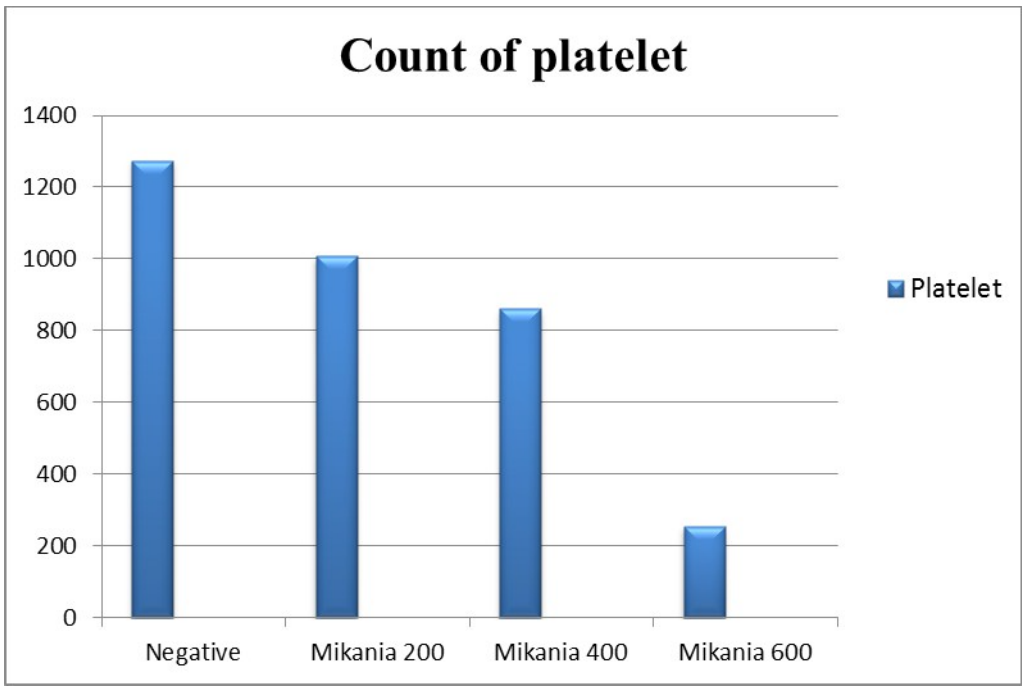
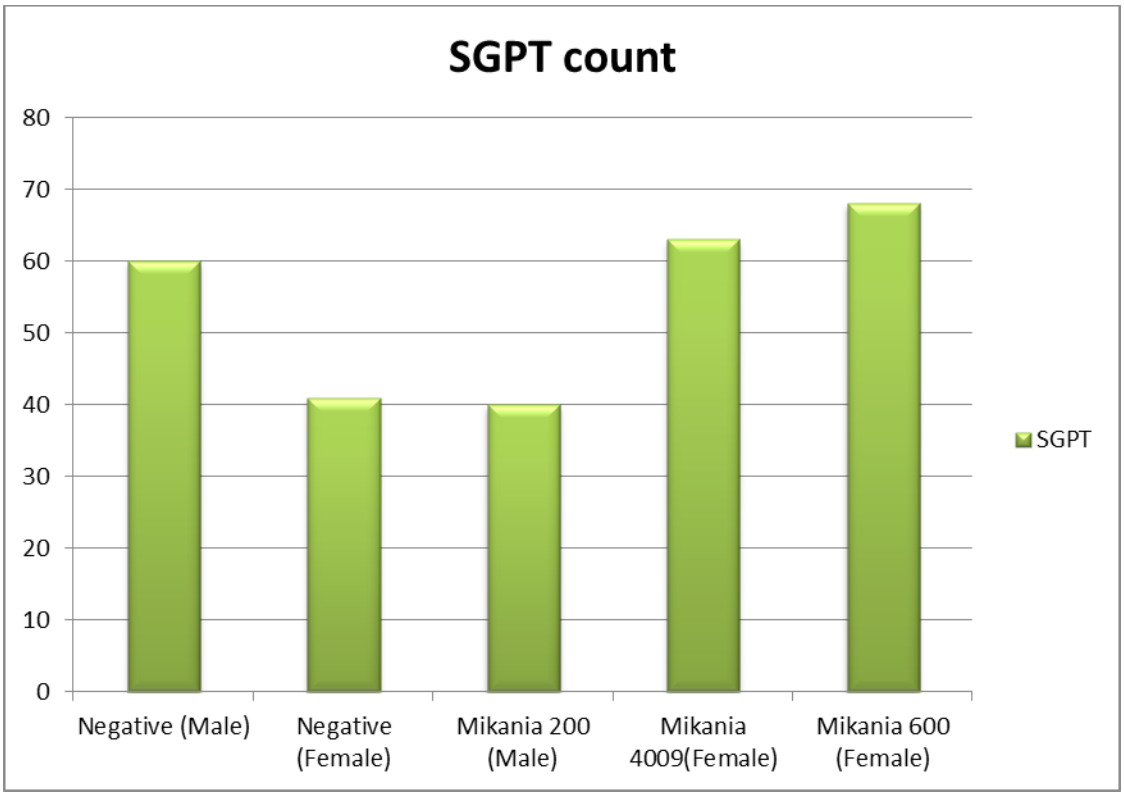


Table-18: Effect of *Mikania cordata* on the Liver Function Test

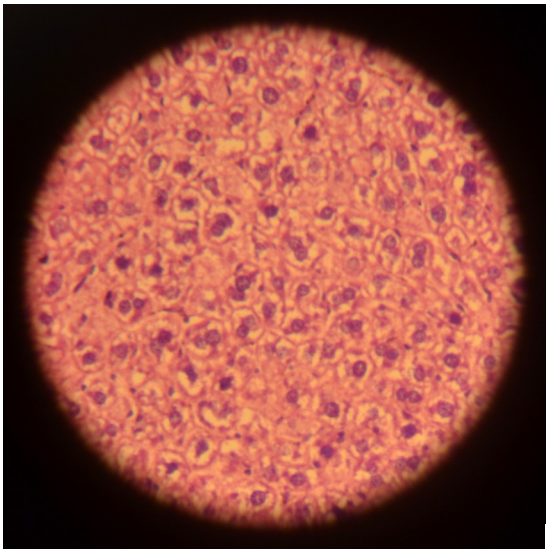
Treatment Group	SGPT (IU/dl) (n)
Negative Control group(5% CMC solution)	60 (Male) 41 (Female)
***Mikania cordata* (200mg/kg)**	40
***Mikania cordata* (400mg/kg)**	63
***Mikania cordata* (600mg/kg)**	68

Figure-44: Effect of *Mikania cordata* on the Liver Function Test

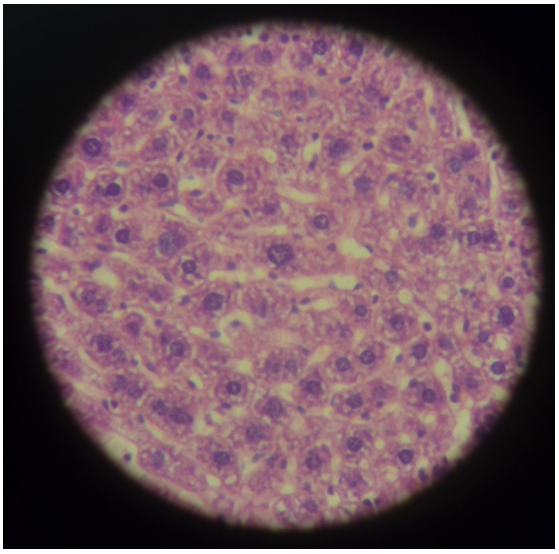


4.4.2.2. Histopathological studies

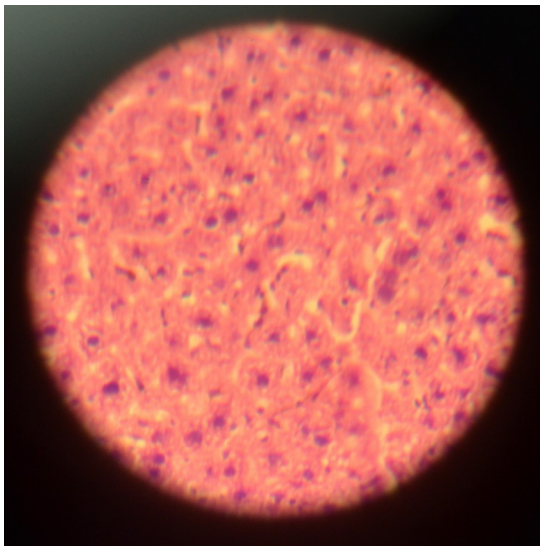
The histological status of the liver tissues of both the treated and control mice where normal cellular architecture with prominent central vain was shown which indicates, that the extract can cause damage to liver if used for therapeutic purpose. This becomes important because liver is the primary organ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



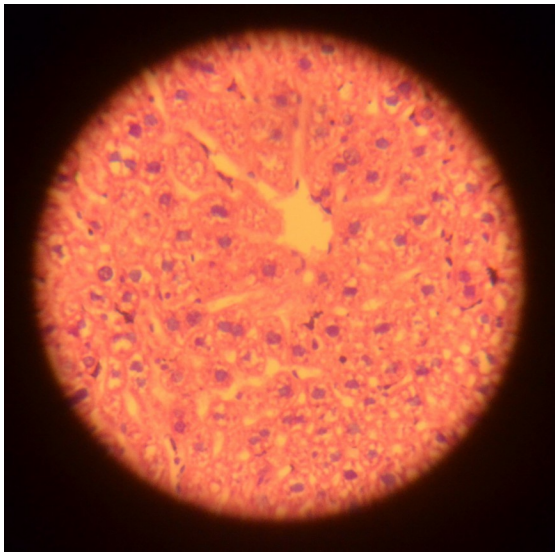
Negative Control



***Mikania cordata* 200mg/kg**



***Mikania cordata* 400mg/kg**



***Mikania cordata* 600mg/kg**

Figure-44: Histopathological test of mice in different group

4.4.3. Abnormalities:

During the total 45 days of sub-chronic toxicity test I observed some abnormalities of mice. These are given below

- One mouse develop eye problem.

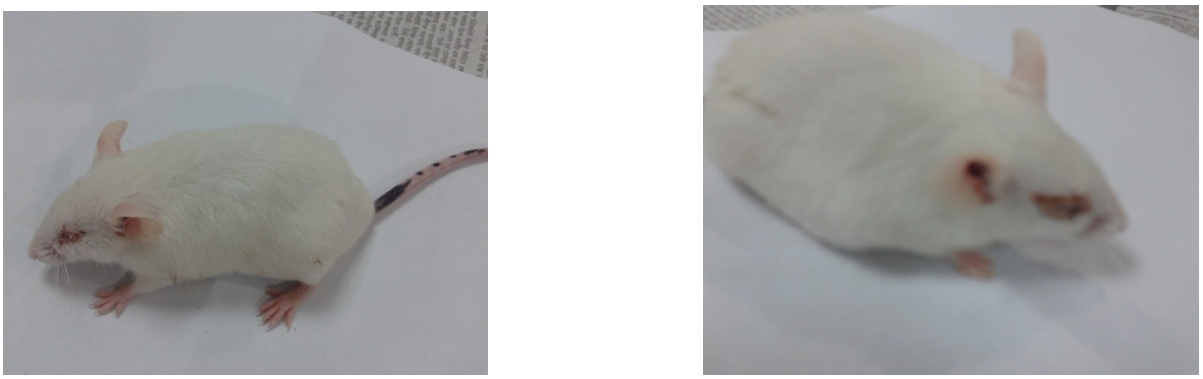


Figure-45: Eye problem of mice

- One mouse develop tail bend.

Figure-46: Tail bending of mice

- One mouse develop tumor in lower abdomen.

In the group of 200mg/kg methalonic extract of *Mikania cordata* develop tumor in the lower abdomen. The CBC Parameters and SGPT result of that mice is given to the next page.

Table-18: CBC and biochemical parameters of tumor mice

Parameters	Total count of WBC	Neutrophil	Lymphocyte	Monocyte	Eosinophil	Basophil
Total count	8.8	15	80	1	3	1
Reference value	3-12 x10 3/ mm3					

Parameters	Total Count of RBC	Haemoglobin	HCT	Mean Corpuscular Volume (MCV)	Mean Corpuscular Haemoglobin (MCH)	Mean Corpuscular Hematocrite Cell (MCHC)	RDW
Total count	7.83	7.8	65.8	84.1	14.6	23.2	19.9
Reference value	7-12 x10 6 /mm3	13-17 g/dl		43-54	13-18	31-34	

Parameters	Total Count of Platelet	MPV	SGPT
Total count	772	7.27	87 U/L
Reference value	1000-1600 x 10 3/ mm3		

4.5. Conclusion

As a part of our project aimed at the pharmacological evaluation of a medicinal plant, I studied the Central Nervous System activities, Analgesic and Anti-inflammatory activities, and Acute

and Subchronic toxicity of methanolic extract of *Mikania cordata.* I also studied Analgesic and Anti-inflammatory activities of different fraction of *Spilanthes acmella.*

The plant extract was also assessed on the central nervous system using a number of neuropharmacological experimental models in mice. The crude extract of *Mikania cordata* (200mg/kg, 400mg/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 reference drug is found slightly potent than the extract. The crude extract of *Mikania cordata* (200mg/kg, 400mg/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 result of the effect of the plant crude extract against acetic acid induced writhing in mice also shown analgesic acitytiv of crude extract and n-hexane, DCM fraction of *Mikania cordata*. The crude extract (800mg/kg, 1200mg/kg) dose dependently reduces the number of abdominal constriction and stretching of hind limb induceded by the injection of acetic acid. The DCM fraction of 500mg/kg significantly reduces the abdominal constriction and stretching of hind limb induceded by the injection of acetic acid while n-hexane fraction of 500mg/kg is negligible. The reduction is significant (***$p<0.001$) when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Indomethacin 10mg/kg .The reference drug is found slightly potent than the crude extract but DCM fraction is more potent than reference drug. The percent inhibition of writhing by groups pretreated with *Mikania cordata* crude (800mg/kg, 1200mg/kg), fraction of DCM-500mg/kg, n-hexane-500mg/kg and Indomethacin is , i.e 52.58%, 62.90%, 84.52%, 27.02%, 72.47% respectively.

The result of the effect of the extract against Formalin induced hind paw licking in mice is shown significant anti-inflammatory activities of crude extract and n-hexane, DCM fraction of *Mikania cordata*. The crude extract (800mg/kg, 1200mg/kg) dose dependently reduces the paw licking time induceded by the injection of formalin. The DCM fraction of 500mg/kg significantly reduces the paw licking time induceded by the injection of formalin while n-hexane fraction of

500mg/kg is negligible. The reduction is significant (***p<0.001) when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Indomethacin 10mg/kg. The reference drug is found slightly potent than the crude extract but DCM fraction is more potent than reference drug. *Mikania cordata* at 800mg/kg, 1200mg/kg and n-hexane-500mg/kg, DCM-500mg/kg doses can reduce nociception in the acute phase in a dose dependent manner 17.09%, 29.50%, 4.97%, 55.16%) but n-hexane is negligible while Aspirin shows significant effect on the acute phase of the formalin test(49.69%). In the delayed phase of formalin test Aspirin produced only 60.45% inhibition, while *Mikania cordata* at 800mg/kg, 1200mg/kg n-hexane-500mg/kg, DCM-500mg/kg doses can reduce inflammation in this phase in a dose dependent manner (38.93%, 55.15%, 5.38%, 65.82%) but n-hexane is negligible while Indomethacin shows significant effect on the acute phase of the formalin test (60.45%). The effect of the crude extract is predominant in the late phase of the formalin induced pain model, suggesting the presence of anti-inflammatory activities.

The aim of the study was also to investigate the possible toxicity of the plant *Mikania cordata* and especially to establish the safety of the aqueous extract of this plant by focusing on its acute and sub-chronic toxicity in mice. For finding sub-chronic toxicity several tests are done such as CBC (Cell Blood count) test, Hepatic enzyme test and histological Studies. CBC test and hepatic enzyme test are done by hematological machine and histological studies by microscopic test. The results of several widely accepted protocols would suggest that there were positive modulations in all the parameters of study in the *Mikania cordata* extract which significant difference were found in RBC and different count of RBC, WBC & different count of WBC values of treated mice. The result shows that increase toxic effect by increase dose such as (200,400 & 600) mg/kg and decrease cell count values and also increase SGPT value. The histological status of the liver tissues of both the treated mice where normal cellular architecture with prominent central vain was shown which indicates that the extract can cause damage to liver if used for maximum dose. This becomes important because liver is the primary organ for detoxification. Also some abnormalities shown in different group which indicates that it is a toxic plant.

The result of the effect of the plant crude extract against acetic acid induced writhing in mice also shown analgesic acitytiv of n-hexane, Chloroform, Ethyl acetate, and water fraction of *Spilanthes acmella*. These fraction of 200mg/kg dose dependently reduces the number of

abdominal constriction and stretching of hind limb induceded by the injection of acetic acid. The Chloroform fraction of 200mg/kg significantly reduces the abdominal constriction and stretching of hind limb induceded by the injection of acetic acid while other fraction is negligible. The reduction is significant (***p<0.001) when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Indomethacin 10mg/kg .The reference drug is found slightly potent than the crude extract but DCM fraction is more potent than reference drug. The percent inhibition of writhing by groups pretreated with n-hexane, Chloroform, Ethyl acetate, and water fraction of *Spilanthes acmella* and Indomethacin is , i.e 54.65%, 81.42%, 43.24%, 29.18%, 62.34% respectively.

The result of the effect of the extract against Formalin induced hind paw licking in mice is shown significant anti-inflammatory activities of n-hexane, Chloroform *Spilanthes acmella*. These fraction of 200mg/kg dose dependently reduces the paw licking time induceded by the injection of formalin. The Chloroform fraction of 200mg/kg significantly reduces the paw licking time induceded by the injection of formalin while other fraction of is negligible. The reduction is significant (***p<0.001) when it is compared to negative control. The effect of the extract is comparable to that of the standard drug, Indomethacin 10mg/kg. The reference drug is found slightly potent than the crude other fraction but Chloroform fraction is more potent than reference drug. *Spilanthes acmella* at n-hexane, Chloroform fraction doses can reduce nociception in the acute phase in a dose dependent manner 8.65%, 25.76%, but n-hexane is negligible while Indomethacin shows significant effect on the acute phase of the formalin test(48.49%). In the delayed phase of formalin test Indomethacin produced only 51.64% inhibition, while n-hexane, Chloroform fraction of *Spilanthes acmella* doses can reduce inflammation in this phase in a dose dependent manner 13.42%, 24.84% but n-hexane is negligible while Indomethacin shows significant effect on the acute phase of the formalin test (51.64%). The effect of the crude extract is predominant in the late phase of the formalin induced pain model, suggesting the presence of anti-inflammatory activities.

From the present investigation, it can be concluded that *Mikania cordata* exhibited depressant activity, excellent analgesic and anti-inflammatory activity and shows toxicity safety in acute and sub-chronic toxicity studies in mice. The study also conclude that the chloroform fraction of

Spilanthes acmella exhibited excellent analgesic and anti-inflammatory activity.

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Annexure

List of Abbreviation	Full Meaning
AAS	Anabolic Androgenic Steroid

AGA	American Gastroenterological Association
ALT	Alanine Transaminase
AST	Aspartate Transaminase
ALP	Alkaline Phosphatase
ANOVA	One-way Analysis of Variance
CAM	Complementary & Alternative Medicine
CBC	Complete Blood Count
CMC	Carboxy Methyl Cellulose
CNS	Central Nervous System
COPD	Chronic Obstructive Pulmonary Disease
COX	Cyclo-oxygenase
DCM	Dichloromethane
DMPP	Descending Modulatory Pain Pathways
DNA	Deoxyribonucleic acid
EDTA	Ethylene Diamine Tetra Acetic acid
EMG	Electromyography
EP	Evoked Potential
EVF	Erythrocyte Volume Fraction
GGT	Gamma-Glutamyl Transferase
GPT	Glutamate Pyruvate Transaminase
HCT	Hematocrit
HIV/AIDS	Human Immunodeficiency Virus Infection and Acquired Immune Deficiency Syndrome
IASP	International Association for the Study of Pain
ICDDR, B	International Centre for Diarrhoeal Disease and Research, Bangladesh
LFTs or LFs	Liver Function Tests
LOX	Lipoxygenase
MCH	Mean Corpuscular Hemoglobin
MCHC	Mean Corpuscular Hemoglobin Concentration
MCV	Mean Cell Volume
MMPI	Minnesota Multiphasic Personality Inventory
MRIs	Magnetic Resonance Imaging's
MS	Multiple sclerosis
NCCAM	National Center for Complementary & Alternative Medicine
NSAID	Non-Steroidal Anti-inflammatory Drug
PAG	Periaqueductal Grey Matter
PCV	Packed Cell Volume
PNS	Peripheral Nervous System
PT	Prothrombin Time
PV	Polycythemia Vera
RBC	Red Blood Cell

RDW or RCDW	Red Blood Cell Distribution Width
RPM	Rotation Per Minute
SALP	Serum Alkaline Phosphatase
SEM	Standard Error Mean
SGOT	Serum Glutamate Oxaloacetate Transaminase
SGPT	Serum Glutamate Pyruvate Transaminase
SPSS	Statistical Package for the Social Science
UDA	Undeca-2*E*-ene-8,10-Diynoic acid Isobutylamide
WBC	White Blood Cell
5-HT	5-hydroxytryptamine