1. INTRODUCTION

1.1 Pain

Pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage”. Pain involves a significant psychological component which can alter its perception and therefore, undergoes extensive processing through the nervous system, and particularly in the brain. Nociception is important for being aware of and reacting to potentially or actually damaging stimuli in the environment. Receptors on specialised sensory neurons mediate the detection of noxious chemical, thermal and mechanical stimuli. To generate a systemic response aimed at self-preservative the electrical signals which are generated at these sites are commonly amplified and transmitted further to the higher centres in the central nervous system (CNS). Pain is a body defense mechanism and is a warning of a problem particularly when it is acute and may become chronic where it outlasts any potential for healing and becomes modified centrally. Pain has adverse effect on well-being in reduced physical and emotion function.

![Figure-1 Physiology of Pain](image.png)
Etymology
First attested in English in 1297, the word pain comes from the Old French peine, in turn from Latin poena, "punishment, penalty" (in L.L. also "torment, hardship, suffering") and that from Greek "ποινή" (poine), generally "price paid", "penalty", "punishment". It also exists in Frisian as "pine" which in turn is related to the English verb "to pine" which means to long for.

Cause of pain
The pain may occur for many reasons. It may be felt because of

- Inflammation
- Infection
- Tissue necrosis
- Stretching of tissue
- Chemicals
- Burn

In skeletal muscle, pain may result from ischemia or haemorrhage. The inflammations of the mucosa or from distension or muscle spasm are the main reasons for pain in the stomach and intestines. Depending on the cause, sudden and short-term, marked primary by a reflex withdrawal.[5]

Pain can be classified as

1. Acute Pain: This is usually of short duration and the cause often identifiable (disease, trauma).
2. Chronic Pain: It persists after healing is expected to be complete, or is caused by a chronic disease.
Figure-2 Classification of Pain

Pain may be modified by psychological factors and attention to these is essential in pain management. [8]

Fast pain is also described by many alternate names such as sharp pain, acute pain, electric pain, and others. The processing of acute and prolonged painful stimulation is depicted which provides the physiological basis for two major characteristics of clinical pain: hyperalgesia and allodynia. An acute stimulus will trigger a series of events leading to excitatory pain signals reaching the brain via the spinal cord; as the stimulus is short lived, so is the neuronal response. However, sensitization may occur at either the peripheral and/or at the central level due to longer and more chronic stimulus. [9]
Peripheral Mechanisms of Sensory Transmission

The sensory experience begins in the periphery, first the peripheral terminals of primary afferent fibres respond to a myriad of stimuli and this information is translated into the dorsal horn of the spinal cord, where the central ends of these fibres terminate. There are three main types of sensory fibre in the peripheral nervous system, Aβ-fibres, Aδ fibres, and C-fibres. Each has different properties allowing them to respond to and transmit different types of sensory information. As Aβ-fibres are large in diameter and highly myelinated, they quickly conduct action potentials from their peripheral to central terminals. These fibres have low activation thresholds and normally respond to light touch and are responsible for conveying tactile information. Aδ fibres are smaller in diameter and thinly myelinated, and they also possess higher activation thresholds. They respond to both thermal and mechanical stimuli. C-fibres are the smallest type of primary afferents and are non-myelinated, thus making them the slowest conducting. They have the highest thresholds for activation and therefore detect selectively nociceptive or painful stimuli. Collectively, both Aδ and C-fibres can be termed as nociceptors or pain fibres, responding to noxious stimuli which may be mechanical, thermal, or chemical.

Chemical Sensitivity of Nociceptors

The results of injury in the local release of neuron chemicals mediate or facilitate the inflammatory process. Such chemical mediators include leukotriene, serotonin, nitric oxide, bradykinin, prostaglandins, histamine, substance P, thromboxane, platelet activating factor, protons and free radicals. Some of these chemicals activate nociceptors and therefore are directly involved in producing pain, while others lead to sensitization of the nociceptors response to natural stimuli and therefore play a role in primary hyperalgesia. Bradykinin is released upon tissue injury, and present in inflammatory exudates. Serotonin can also potentiate the pain induced by bradykinin and enhance the response of nociceptors to bradykinin. Mast cells, upon degranulation release platelet activating factor which in turn lead to serotonin release from platelets. Protons selectively activate nociceptors and produce a sensitization of nociceptors to mechanical stimuli. Histamine can lead to a variety of response including vasodilation and oedema. Substance P is released from nociceptors terminals and it causes the release of histamine from mast cells. Arachidonic acid metabolites (prostaglandins, thromboxanes and leukotriene’s) are collectively known as eicosanoids. The eicosanoids are generally considered not to active nociceptors. This sensitizing effect of eicosanoids may play an important role in hyperalgesia associated with inflammation.
Pain-Producing (Algesic) Substance
Algesic substances are released by damaged tissues, either directly or indirectly evoke pain, including substances such as H+, K+, acetylcholine, histamine, serotonin (5-HT), bradykinin which can directly stimulate the sensory nerve endings. This phenomenon is known as peripheral sensitization.\[10]\n
Pathophysiology of Pain
The chemical messengers released during tissue injury stimulate nociceptors to release neuropeptides such as substance P, neurokinin A, calcium gene-related peptides and nerve growth factor. These neuropeptides lead to peripheral sensitization. Under the guidance of the sympathetic nervous system after painful injury, the release of norepinephrine stimulates release of excitatory transmitters at the level of the spinal cord. These excitatory transmitters have been implicated in the process of central sensitization and work to prolong the response of dorsal horn neurons, resulting in persistent changes in the excitability of the cell, also referred to “wind-up”. Central sensitization refers to sensory changes in the undamaged tissue surrounding the injury owing to hypersensitive spinal neurons, which produce secondary pain. Tenderness in a zone surrounding the injury is due to central sensitization. Nociception is conveyed from the periphery to the brain by an adaptable and dynamic pathway. The pathway is transmitted and modulated at three levels: the peripheral nociceptors, the spinal (dorsal horn of the cord) and the supraspinal (brain).\[12]\n
Peripheral Sensitization
Neurons that have a bipolar structure conduct the nociceptive information from the periphery to the CNS. The cell body lies within the dorsal root ganglion (DRG), with the proximal axon running into the dorsal aspect of the spinal cord. Due to the uneven distribution of ions within and outside the cell membrane, the resting membrane potential of these cells is 50-100 mV. An adenosine triphosphates (ATPase) pump continuously keeps the concentration of sodium outside the cell at 20 times of that within the cell. Conversely, this pumping system maintains a potassium concentration inside the cell at 35 times of that outside, charge becomes either neutral or negative. This result in an action potential and it can only run in one direction due to the refractory phase that follows membrane depolarization in nociceptors that is from the periphery towards the spinal cord. There are two types of nociceptors: fast conducting A\(\delta\) fibres and the more slowly conducting C fibres. A\(\delta\) fibres associated with cold and pressure, and as nociceptors they convey
fast pain information. They are thinly myelinated, so conduct signals more rapidly than non-myelinated C fibres. These fibres are rapid in transfer of information from the periphery to the spinal cord, and these fibres are high-threshold receptors which respond to mechanical stimulation such as firm pinch. Some of the fibres also respond to noxious heat (> 45°C). Aδ fibres activation result in sharp pain. C fibres are non-myelinated unlike most other fibres in the nervous system. This lack of myelination is the cause of their slow conduction velocity, which is on the order of no more than 2m/s. C fibres are on average 0.2-1.5μm in diameter. The other main classification of nociceptors is Aδ fibres. These fibres have axons that are larger (1-5μm), in diameter, are myelinated, and have a higher conduction velocity, which is on the order of about 20m/s. Overall, the C-fibre population is responsible for 80% of the nociceptive primary afferents and the perception of this information tends to be associated with poorly localized, aching and burning pain.

When the tissue injury occurs, potassium and kinins are released from the damage cells. These substances stimulate the receptor directly, resulting in the release of neuropeptides such as substance P from the receptor. This in turn causes the degranulation of adjacent mast calls with the production of platelet-activating factor (PAF) which in turn releases serotonin from the platelets. Histamine is also released from the mast call, starting an inflammatory reaction within the tissue with vasodilation, lowered P\text{H} and the release of eicosanoids such as leukotriene’s and prostaglandins (PGs).\[^{13}\]

**Central Sensitization**

Nociceptive formation within the Aδ and C fibres arrives at the dorsal horn of spinal cord. C fibres terminate in the superficial dorsal horn at the laminar II, and Aδ fibres terminate in the laminar I and V. Aδ fibres terminate mainly in the laminar I and V with some of their high-threshold fibres ending directly in laminar II.

Most cutaneous C fibres terminate in the laminae II. However, visceral C fibres terminate in the laminar I, II, IV, V, and X. Peripheral nerve injury leads to an increase in the general excitability of multi-receptive spinal cord neurons. This hyper excitability is manifested by increased neuronal activity in response to noxious stimuli, expansion of neuronal receptive fields and spreads of a spinal hyper excitability to other segments.\[^{14}\]
The results of C fibres are the increased dorsal horn excitatory. C fibres release not only glutamate but also substance P, which acts through the neurokinin-1 (NK-1) receptor to increase dorsal horn intracellular calcium and enhance N-methyl D-aspartate (NMDA) sensitivity to glutamate. When primary afferent neuron release glutamate, this glutamate binds to NMDA and there is an influx of Ca\(^{2+}\) into the post synaptic neuron. These results could activate enzyme such as nitric oxide (NO) synthases or trigger other long lasting cellular changes, so signal transduction comes to sensory projection fields in the cortex. This part of the cortex is responsible for the conscious perception of pain and particularly localizing and registering the intensity of the pain. The ascending reticular activating system has an influence on evolution. This state of hyper excitability is called central sensitization.

Figure-3 Sensitization of Pain Process
Figure-4 Site of Action of Various Analgesic Drugs
1.2 Inflammation

Inflammation is the body’s response to tissue injury. Inflammation is a defence reaction of the organism and its tissue to injurious stimuli that lead to the local accumulation of plasmatic fluid and blood cells. Although it is a defence mechanism, the complex events and mediators involved in the inflammatory reaction can be included, maintained or aggravated by many diseases.

Causes for inflammation

Causes may include

- Direct damage such as cuts, sprains etc.
- Chemicals such as acid
- Ischemia
- Cell necrosis
- Allergic reactions
- Physical agents such as thermal injuries or burns

Classification of Inflammation

![Figure-5 Physiological Processes in Acute and Chronic Inflammation](image-url)
1. **Acute Inflammation**

Acute inflammation begins within seconds to minutes following the injury of tissues. The damage may be purely physical, or it may involve the activation of an immune response. Three main processes occur:

- Increased blood flow due to dilation of blood vessels (arterioles) **supplying the region**.
- Increased permeability of the capillaries, allowing fluid and blood proteins to move into the interstitial spaces.
- Migration of neutrophils (and perhaps a few macrophages) out of the venules and into interstitial spaces.

The cardinal signs of acute inflammation

1. **Redness (Rubor)**: An acute inflamed tissue appears red, for example skin affected by sunburn.

2. **Heat (Calor)**: Increase of temperature on the skin is seen only in peripheral parts of the body. It is due to increased blood flow to the region, resulting in vascular dilation and the delivery of warm blood to the area.

3. **Swelling (Tumor)**: Swelling result from edema due to accumulation of fluid in the extra vascular space as a part of the fluid exudates.

4. **Pain (Dolor)**: It results partly from the stretching and distortion of tissue due to inflammatory edema and, in particular, from pus under pressure in an abcess cavity.

5. **Loss of Function**: It is a well-known consequence of inflammation. Movement of an inflamed area is consciously and reflex inhibited by pain.

**Process of Acute Inflammation**

The process of acute inflammation is initiated by cells already present in all tissues, mainly resident macrophages, dendritic cells, histiocytes, kupffer cells and mastocytes. At the onset of an infection, burn, or other injuries, these cells undergo activation and release inflammatory mediators responsible for the clinical signs of inflammation. Vasodilation and its resulting increased blood flow cause, the redness (rubor) and increased heat (calor). Increased permeability of the blood
vessels, results in an exudation (leakage) of plasma proteins and fluid into the tissue (edema), which manifests itself as swelling (tumor). Some of the released mediators such as bradykinin increase the sensitivity to pain (hyperalgesia). The mediator molecules also alter the blood vessels to permit the migration of leukocytes, mainly neutrophils, outside of the blood vessels (extravasation) into the tissue. The neutrophils migrate along a chemotactic gradient created by the local cells to reach the site of injury. The loss of function is probably the result of a neurological reflex in response to pain. In addition to cell-derived mediators, several biochemical cascade systems consisting of preformed plasma proteins act in parallel to initiate and propagate the inflammatory response. These include the complement system activated by bacteria, and the coagulation and fibrinolysis systems activated by necrosis, e.g. a burn or a trauma.

The acute inflammatory response requires constant stimulation to be sustained. Inflammatory mediators have short half-lives and are quickly degraded in the tissue. Hence, inflammation ceases once the stimulus has been removed.

**Figure-6 Process Events in Inflammation**
**Chemical Mediators of Inflammation**

- Histamine is the main pre-formed mediator of inflammation. Released from mast cells, basophils, and platelets, it cause transient dilatation of arterioles, increases permeability in venules, and is the primary cause of increased vascular permeability in the first hour after injury.

- Prostaglandins: Released from mast cells. It is derived by local synthesis by Arachidonic acid. A group of lipids which can cause vasodilation, fever, and pain.

- Leukotriene B$_4$ is able to mediate leukocyte adhesion and activation, allowing them to bind to the endothelium and migrate across it. In neutrophils, it is also a potent chemo attractant, and is able to induce the formation of reactive oxygen species and the release of lysosome enzymes by these cells.

- Nitric oxide is a small molecule that is locally synthesized by endothelium and macrophages through the activity of the enzyme, nitric oxide synthase.

![Chemical mediators involved in inflammation process](image)

**Figure-7** Chemical mediators involved in inflammation process NO in acute inflammation
A variety of chemical mediators, operating sequentially in different phases of the inflammatory response, mediate the associated changes in micro vascular calibre and permeability. In addition, cell injury/necrosis and neutrophil infiltration are also principal changes. NO is inhibited in the intermediate phase of vasodilatation and increased vascular permeability in skin inflammation after injection of carrageenan or exposure to ultraviolet light, suggesting that NO involved in skin inflammation of some hours duration. However, it is unclear whether NO has a role in the immediate acute phase of inflammation; it is possible that the transitory initial vasoconstriction seen initially may be due, at least in part, to an acute loss of endothelial NO synthesis as a result of cell dysfunction. Thus, the increase in vascular permeability is a key, early event in an acute inflammatory process.\textsuperscript{[21]}

**NO in Chronic Inflammation**

Macrophage infiltration, proliferative and synthetic connective tissue reactions, and, in processes where hypersensitivity is involved, T lymphocyte accumulation are all regarded as the hallmarks of chronic inflammation. Usually, macrophage infiltration is demonstrated by processes similar to those for neutrophils acting on circulating blood monocytes, but with certain crucial differences in the chemo attractants and adhesion molecules involved. In addition, the response is highly dependent on the release of cytokines.\textsuperscript{[21]}

- Platelet activating factor (PAF) is synthesized by mast cell or basophils and can be stimulated by IgE-mediated release. It is a specialized phospholipids compound, which causes vasoconstriction, increased vascular permeability and platelet aggregation.
- Cytokines are polypeptide products of activated lymphocytes and monocytes. The main cytokines participating in acute inflammation are interleukin-1 (IL-1), interleukin-8 (IL-8), and tumour necrosis factor alpha (TNF\textsubscript{\alpha}).

The chemokine’s are a family of factors secreted by leukocytes and endothelial cells in response to tissue damage and other inflammatory mediators. Specific chemokine receptors are activated and this signal for activation of leukocyte integrin’s, mediating adhesion and migration.

**The complement System**

There is a cascade of activation, with production of numerous intermediary activated peptides. The main products with roles in acute inflammation are as follow:
- C3a increases vascular permeability by liberating histamine from mast cells or platelet.
- C5a stimulates histamine release by mast cells, thereby producing vasodilation. It is also able to act as a chemo attractant to direct cells via chemotaxis to the site of inflammation.
- C345 is chemotactic to neutrophils.
- C3b opsonizes bacteria and facilitates neutrophils phagocytosis.
- The kinins are small peptides derived from plasma precursors by proteolytic cleavage. The system is activated by one of the coagulation proteins, activated Hageman factor (factor XII). \[22\]
- The clotting pathway is responsible for coagulation of blood by formation of fibrin from fibrinogen. Factor XII is activated the inflammatory exudates when it comes into with collagen outside the vessel. These cause increased vascular permeability, as well as being chemotactic for neutrophils.
- The thrombolytic pathway: The enzyme plasmin is a proteolytic enzyme with several roles in inflammation.

**Pathophysiology of Acute Inflammation**

The acute response to tissue injury occurs in the microcirculation at the site of injury. Initially there is a transient constriction of arterioles; however, within several minutes, chemical mediators released at the site cause relaxation of arteriolar smooth muscle, vasodilation, and increased capillary permeability. Protein-rich fluid then exudes from capillaries into the interstitial space. This fluid contains many of the components of plasma, including fibrinogen, kinins, complement, and immunoglobulin’s that mediate the inflammatory response. The sub-acute phase is characterized by movement of phagocytic cells to the site of injury. In response to adhesion molecules released from activated endothelial cells, leukocytes, platelets and RBC in injured vessels become sticky and adhere to the endothelial cell surfaces.

Polymorph nuclear leukocytes such as neutrophils are the first cells to infiltrate the site of injury. Basophils and eosinophil’s are more prevalent in allergic reactions or parasitic infections. As the inflammatory process continues, macrophages predominate, actively removing damaged cells or tissue. If the cause of injury is eliminated, acute inflammation may be followed by a period of tissue repair. Blood clots are removed by fibrinolysis, and damaged tissues are regenerated or
replaced with fibroblasts, collagen, or endothelial cells. However, inflammation may become chronic, leading to further tissue destruction and fibrosis.\[^{5}\]

**Acute Inflammation Flow Chart**

**STIMULI**
- Infection (bacterial, viral, parasitic) or microbial toxins
- Trauma (blunt or penetrating)
- Physical (e.g., temperature, irradiation) or chemical agents
- Tissue necrosis (from any cause)
- Foreign bodies (e.g., splinters, dirt, suture)
- Immune reactions (hypersensitivity)

**Chemical Mediators:**
- Vasoactive amines
- C3a
- Bradykinin
- LT(C4, D4, E4

**Vessel Permeability**
- Venules
- Via phosphorylation of contractile proteins (causing endothelial cell contraction)

**Mast Cells Degranulate**
- Histamine; PG's; Serotonin

**Endothelial Cells**
- Nitric Oxide

**VASODILATION** (arterioles)
- Relaxation of vascular smooth muscle

**Rubor (Redness)**
- Calor (Heat)

**↑ Vessel CALIBRE & FLOW**

**↑ Hydrostatic Pressure (Pc), ↓ TRANSDUATE (low protein)**

** Fluid to Interstitial Spaces**

**↓ Blood Flow**

**Tumor (œdema)**

**Margination & Adhesion of LEUKOCYTES**

**Transmigration** (diapedesis)

**Migration/ Chemotaxis**

**Leukocyte Activation**
- Recognition of opsonins (IgG, C3b, mannose binding lectin)
- Phagocytosis; reactive oxygen species, lysosomal enzymes, etc

**Termination**
- Mediator breakdown
- Removal of stimulus
- Anti-inflammatory mediators

**Figure-8** Flow Chart Depicting Acute Inflammation
2. Chronic Inflammation

Chronic Inflammation has a slow onset and persists for weeks or more. The symptoms are not as severe as with acute inflammation, but the condition is insidious and persistent. Chronic inflammation may follow on from acute inflammation or exist by itself. An acute inflammation will become chronic if the immune system is unable to rid the body of the offending foreign agent or if the agent is constantly able to re-enter the body. In the case of persistent infections, such as tuberculosis, and autoimmune diseases, chronic fatigue will arise without the person first going through the acute inflammation stage. The main cells involved in chronic infection are macrophages and lymphocytes. Because both these cells have a single nucleus, they are known as mononuclear cells. With the aid of chemical mediators such as lymphokines, macrophages do an excellent job of engulfing and neutralizing or killing foreign antigens. Lymphocytes are the predominant cell in chronic inflammation. There are two types, labelled T and B. T-lymphocytes are produced in the thymus gland. They ensure cell based immunity from infection. B-lymphocytes originate in the bone marrow and ensure humoral (bodily fluid) immunity. The activation of B-lymphocytes produces plasma cells, which manufacture and secrete antibodies to fight specific types of antigens. Macrophages and lymphocytes are interdependent that the activation of one stimulates the actions of the other. In certain chronic infections cells known as eosinophils get accumulated. Within their cytoplasm are bright red granules. These granules contain a substance called ‘major basic protein’ which has the ability to destroy certain antigens. In cases of chronic inflammation involving foreign particulate matter, such as splinters, macrophages cells can fuse together to form multinucleated giant cells. Tuberculosis may also cause macrophage cells to unite in this manner. A key feature of chronic inflammation is collagen production. If too much collagen is formed, this can lead to the condition known as fibrosis. Connective tissue cells known as fibroblasts enter the area of tissue injury and then go to work to produce collagen which is necessary to replace the tissue lost during long term inflammation. The dilated blood vessels which are characteristic of acute inflammation are not evident in cases of chronic inflammation. The two major complications associated with chronic infection are fibrosis leading to scarring and persistence. The overabundance of collagen production over time can lead to scarring that can cause permanent distortion of the tissue, interfering with its function. Chronic inflammation can be continually stimulated by substances with low antigenic properties or by auto-immunity.
Pathophysiology of Chronic Inflammation

Characteristics of chronic inflammation include less swelling but the presence of more lymphocytes, and fibroblasts than in acute inflammation and macrophage have been unable to completely clear (debride) the area of foreign substances. This material may be dead cells, extracellular blood, or sand or dirt in some cases. Either way, the material is surrounded by collagen to isolate it from the body. This mass of encapsulating scar is called a granuloma.\[23\]
**Table 1: Plasma derived mediators**

<table>
<thead>
<tr>
<th>Name</th>
<th>Produced by</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin</td>
<td><em>Kinin system</em></td>
<td>A vasoactive protein which is able to induce vasodilation, increase vascular permeability, cause smooth muscle contraction, and induce pain.</td>
</tr>
<tr>
<td><strong>C3</strong></td>
<td><em>Complement system</em></td>
<td>Cleaves to produce <em>C3a</em> and <em>C3b</em>. <em>C3a</em> stimulates histamine release by mast cells, thereby producing vasodilation. <em>C3b</em> is able to bind to bacterial cell walls and act as an opsonin, which marks the invader as a target for phagocytosis.</td>
</tr>
<tr>
<td>C5a</td>
<td><em>Complement system</em></td>
<td>Stimulates histamine release by mast cells, thereby producing vasodilation. It is also able to act as a chemo attractant to direct cells via chemotaxis to the site of inflammation.</td>
</tr>
<tr>
<td>Factor XII (Hageman Factor)</td>
<td><em>Liver</em></td>
<td>A protein which circulates inactively, until activated by collagen, platelets, or exposed basement membranes via conformational change. When activated, it in turn is able to activate three plasma systems involved in inflammation: the kinin system, fibrinolysis system, and coagulation system.</td>
</tr>
<tr>
<td>Membrane Attack Complex</td>
<td><em>Complement system</em></td>
<td>A complex of the complement proteins C5b, C6, C7, C8, and multiple units of C9. The combination and activation of this range of complement proteins forms the <em>membrane attack complex</em>, which is able to insert into bacterial cell walls and causes cell lysis with ensuing death.</td>
</tr>
<tr>
<td>Plasmin</td>
<td><em>Fibrinolysis system</em></td>
<td>Able to break down fibrin clots, cleave complement protein C3, and activate Factor XII.</td>
</tr>
<tr>
<td>Thrombin</td>
<td><em>Coagulation system</em></td>
<td>Cleaves the soluble plasma protein fibrinogen to produce insoluble fibrin, which aggregates to form a blood clot. Thrombin can also bind to cells via the PPAR1 receptor to trigger several other inflammatory responses, such as production of chemokine’s and nitric oxide.</td>
</tr>
</tbody>
</table>
## Table 2: Cell derived mediators

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Source</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lysosome Granules</strong></td>
<td><strong>Enzymes</strong></td>
<td>Granulocytes</td>
<td>These cells contain a large variety of enzymes which perform a number of functions. Granules can be classified as either <em>specific</em> or <em>azurophilic</em> depending upon the contents, and are able to break down a number of substances, some of which may be plasma-derived proteins which allow these enzymes to act as inflammatory mediators.</td>
</tr>
<tr>
<td><strong>Histamine</strong></td>
<td><strong>Vasoactive amine</strong></td>
<td>Mast cells, basophils, platelets</td>
<td>Stored in preformed granules, histamine is released in response to a number of stimuli. It causes arteriole dilation and increased venous permeability.</td>
</tr>
<tr>
<td><strong>IFN-γ</strong></td>
<td><strong>Cytokine</strong></td>
<td>T-cells, NK cells</td>
<td>Antiviral, immunoregulatory, and anti-tumour properties. This interferon was originally called macrophage-activating factor, and is especially important in the maintenance of chronic inflammation.</td>
</tr>
<tr>
<td><strong>IL-8</strong></td>
<td><strong>Chemokine</strong></td>
<td>Primarily macrophages</td>
<td>Activation and chemo attraction of neutrophils, with a weak effect on monocytes and eosinophils.</td>
</tr>
<tr>
<td><strong>PGs</strong></td>
<td><strong>Eicosanoid</strong></td>
<td>Mast cells</td>
<td>A group of lipids which can cause vasodilation, fever, and pain.</td>
</tr>
<tr>
<td><strong>LTB4</strong></td>
<td><strong>Eicosanoid</strong></td>
<td>Leukocytes</td>
<td>Able to mediate leukocyte adhesion and activation, allowing them to bind to the endothelium and migrate across it. In neutrophils, it is also a potent chemo attractant, and is able to induce the formation of reactive oxygen species and the release of lysosome enzymes.</td>
</tr>
</tbody>
</table>
enzymes by these cells.

<table>
<thead>
<tr>
<th>NO</th>
<th>Soluble gas</th>
<th>Macrophages, endothelial cells, some neurons</th>
<th>Potent vasodilator, relaxes smooth muscle, reduces platelet aggregation, aids in leukocyte recruitment, direct antimicrobial activity in high concentrations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α and IL-1</td>
<td>Cytokines</td>
<td>Primarily macrophages</td>
<td>Both affect a wide variety of cells to induce many similar inflammatory reactions: fever, production of cytokines, endothelial gene regulation, chemotaxis, leukocyte adherence, activation of fibroblasts. Responsible for the systemic effects of inflammation, such as loss of appetite and increased heart rate. TNF-alpha inhibits osteoblast differentiation.</td>
</tr>
</tbody>
</table>

1.3 CANCER

Cancer medically known as a malignant neoplasm, is a broad group of various diseases, all involving unregulated cell growth. In cancer, cells divide and grow uncontrollably, forming malignant tumours and invade nearby parts of the body. The cancer may also spread to more distant parts of the body through the lymphatic system or blood stream. Not all tumours are cancerous. Benign tumours do not grow uncontrollably, do not invade neighbouring tissues, and do not spread throughout the body. There are over 200 different known cancers that afflict humans. Determining what causes cancer is complex. Many things are known to increase the risk of cancer, including tobacco use, certain infections, radiation, lack of physical activity, obesity and environmental pollutants. These can directly damage genes or combine with existing genetic faults within cells to cause the disease. Approximately 5-10% of cancers are entirely hereditary.

Cancer can be detected in a number of ways, including the presence of certain signs and symptoms, screening tests, or medical imaging. Once a possible cancer is detected it is diagnosed by microscopic examination of a tissue sample. Cancer is usually treated with chemotherapy, radiation therapy and surgery. The chances of surviving the disease vary greatly by the type and location of the cancer and the extent of disease at the start of treatment. While cancer can affect
people of all ages, and a few types of cancer are more common in children, the risk of developing cancer generally increases with age. In 2007, cancer caused about 13% of all human deaths worldwide (7.9 million). Rates are rising as more people live to an old age and as mass lifestyle changes occur in the developing world. [26]

**Epidemiology**

In 2008 approximately 12.7 million cancers were diagnosed (excluding non-melanoma skin cancers and other non-invasive cancers) and 7.6 million people died of cancer worldwide. Cancers as a group account for approximately 13% of all deaths each year with the most common being, lung cancer (1.4 million deaths), stomach cancer (740,000 deaths), liver cancer (700,000 deaths), colorectal cancer (610,000 deaths), and breast cancer (460,000 deaths). [27] This makes invasive cancer the leading cause of death in the developed world and the second leading cause of death in the developing world. Over half of cases occur in the developing world.

**Prognosis**

Cancer has a reputation as a deadly disease. Taken as a whole, about half of people receiving treatment for invasive cancer (excluding carcinoma in situ and non-melanoma skin cancers) die from cancer or its treatment. Survival is worse in the developing world. However, the survival rates vary dramatically by type of cancer, with the range running from basically all people surviving to almost no one surviving.

Those who survive cancer are at increased risk of developing a second primary cancer at about twice the rate of those never diagnosed with cancer. The increased risk is believed to be primarily due to the same risk factors that produced the first cancer, partly due to the treatment for the first cancer, and potentially related to better compliance with screening.

Predicting either short-term or long-term survival is difficult and depends on many factors. The most important factors are the particular kind of cancer and the patient's age and overall health. People who are frail with many other health problems have lower survival rates than otherwise healthy people.
A centenarian is unlikely to survive for five years even if the treatment is successful. People who report a higher quality of life tend to survive longer. People with lower quality of life may be affected by major depressive disorder and other complications from cancer treatment and/or disease progression that both impairs their quality of life and reduces their quantity of life. Additionally, patients with worse prognoses may be depressed or report a lower quality of life directly because they correctly perceive that their condition is likely to be fatal.

**Research**
Because cancer is a class of diseases, it is unlikely that there will ever be a single "cure for cancer" any more than there will be a single treatment for all infectious diseases. Angiogenesis inhibitors were once thought to have potential as a "silver bullet" treatment applicable to many types of cancer, but this has not been the case in practice.

Experimental cancer treatments are treatments that are being studied to see whether they work. Typically, these are studied in clinical trials to compare the proposed treatment to the best existing treatment. They may be entirely new treatments, or they may be treatments that have been used

*Figure-10 Prognosis of Cancer*
successfully in one type of cancer, and are now being tested to see whether they are effective in another type. More and more, such treatments are being developed alongside companion diagnostic tests to target the right drugs to the right patients, based on their individual biology. Cancer research is the intense scientific effort to understand disease processes and discover possible therapies.

Research about cancer causes focuses on the following issues:

- Agents (e.g. viruses) and events (e.g. mutations) which cause or facilitate genetic changes in cells destined to become cancer.
- The precise nature of the genetic damage, and the genes which are affected by it.
- The consequences of those genetic changes on the biology of the cell, both in generating the defining properties of a cancer cell, and in facilitating additional genetic events which lead to further progression of the cancer.

![Figure-11 Process of Cancer Cause](image-url)
The improved understanding of molecular biology and cellular biology due to cancer research has led to a number of new treatments for cancer since U.S. President Nixon declared the "War on Cancer" in 1971. Since then, the U.S. has spent over $200 billion on cancer research, including resources from the public and private sectors and foundations. During that time, the country has seen a five present decrease in the cancer death rate (adjusting for size and age of the population) between 1950 and 2005.

**Cell Physiology**
Treating cells with the cytotoxic compound can result in a variety of cell fates. The cells may undergo necrosis, in which they lose membrane integrity and die rapidly as a result of cell lysis. The cells can stop actively growing and dividing (a decrease in cell viability), or the cells can activate a genetic program of controlled cell death (apoptosis).

Cells undergoing necrosis typically exhibit rapid swelling, lose membrane integrity, shut down metabolism and release their contents into the environment. Cells that undergo rapid necrosis in vitro do not have sufficient time or energy to activate apoptotic machinery and will not express apoptotic markers. Apoptosis is characterized by well-defined cytological and molecular events including a change in the refractive index of the cell, cytoplasmic shrinkage, nuclear condensation and cleavage of DNA into regularly sized fragments. Cells in culture that are undergoing apoptosis eventually undergo secondary necrosis. They will shut down metabolism, lose membrane integrity and lyse.

![Figure-12 Development of Cancer Cells](image-url)
Signs and Symptoms

Symptoms of cancer metastasis depend on the location of the tumor. When cancer begins, it invariably produces no symptoms with signs and symptoms only appearing as the mass continues to grow or ulcerates. The findings of that result depend on the type and location of the cancer. Few symptoms are specific, with many of them also frequently occurring in individuals who have other conditions. Cancer is the new "great imitator". Thus it is not uncommon for people diagnosed with cancer to have been treated for other diseases to which it was assumed their symptoms were due.

Local Effects

Local symptoms may occur due to the mass of the tumour or its ulceration. For example, mass effects from lung cancer can cause blockage of the bronchus resulting in cough or pneumonia; oesophageal cancer can cause narrowing of the oesophagus, making it difficult or painful to swallow; and colorectal cancer may lead to narrowing or blockages in the bowel, resulting in changes in bowel habits. Masses of breast or testicles may be easily felt. Ulceration can cause bleeding which, if it occurs in the lung, will lead to coughing up blood, in the bowels to anaemia or rectal bleeding, in the bladder to blood in the urine, and in the uterus to vaginal bleeding. Although localized pain may occur in advanced cancer, the initial swelling is usually painless. Some cancers can cause build-up of fluid within the chest or abdomen.

Systemic Symptoms

General symptoms occur due to distant effects of the cancer that are not related to direct or metastatic spread. These may include unintentional weight loss, fever, being excessively tired, and changes to the skin. Hodgkin´s disease, leukemias, and cancers of the liver or kidney can cause a persistent fever of unknown origin.

Specific constellations of systemic symptoms, termed Para neoplastic phenomena, may occur with some cancers. Examples include the appearance of myasthenia gravis in thymoma and clubbing in lung cancer.

Metastasis

Symptoms of metastasis are due to the spread of cancer to other locations in the body. They can include enlarged lymph nodes (which can be felt or sometimes seen under the skin and are
typically hard), hepatomegaly (enlarged liver) or splenomegaly (enlarged spleen) which can be felt in the abdomen, pain or fracture of affected bones, and neurological symptoms.

**Causes**
Cancers are primarily an environmental disease with 90–95% of cases attributed to environmental factors and 5–10% due to genetics. *Environmental*, as used by cancer researchers, means any cause that is not inherited genetically, not merely pollution. Common environmental factors that contribute to cancer death include tobacco (25–30%), diet and obesity (30–35%), infections (15–20%), radiation (both ionizing and non-ionizing, up to 10%), stress, lack of physical activity, and environmental pollutants. It is nearly impossible to prove what caused a cancer in any individual, because most cancers have multiple possible causes. For example, if a person who uses tobacco heavily develops lung cancer, then it was probably caused by the tobacco use, but since everyone has a small chance of developing lung cancer as a result of air pollution or radiation, then there is a small chance that the cancer developed because of air pollution or radiation.

**Pathophysiology**
Cancers are caused by a series of mutations. Each mutation alters the behaviour of the cell somewhat. Cancer is fundamentally a disease of failure of regulation of tissue growth. In order for a normal cell to transform into a cancer cell, the genes which regulate cell growth and differentiation must be altered. The affected genes are divided into two broad categories. Oncogenes are genes which promote cell growth and reproduction. Tumor suppressor genes are genes which inhibit cell division and survival. Malignant transformation can occur through the formation of novel oncogenes, the inappropriate over-expression of normal oncogenes, or by the under-expression or disabling of tumor suppressor genes. Typically, changes in many genes are required to transform a normal cell into a cancer cell. Genetic changes can occur at different levels and by different mechanisms. The gain or loss of an entire chromosome can occur through errors in mitosis. More common are mutations, which are changes in the nucleotide sequence of genomic
Large-scale mutations involve the deletion or gain of a portion of a chromosome. Genomic amplification occurs when a cell gains many copies (often 20 or more) of a small chromosomal locus, usually containing one or more oncogenes and adjacent genetic material. Translocation occurs when two separate chromosomal regions become abnormally fused, often at a characteristic location. A well-known example of this is the Philadelphia chromosome, or translocation of chromosomes 9 and 22, which occurs in chronic myelogenous leukemia, and results in production of the BCR-ABL fusion protein, an oncogenic tyrosine kinase.

Small-scale mutations include point mutations, deletions, and insertions, which may occur in the promoter region of a gene and affect its expression, or may occur in the gene's coding sequence and alter the function or stability of its protein product. Disruption of a single gene may also result from integration of genomic material from a DNA virus or retrovirus, and resulting in the expression of viral oncogenes in the affected cell and its descendants.
Replication of the enormous amount of data contained within the DNA of living cells will probabilistically result in some errors (mutations). Complex error correction and prevention is built into the process, and safeguards the cell against cancer. If significant error occurs, the damaged cell can "self-destruct" through programmed cell death, termed apoptosis. If the error control processes fail, then the mutations will survive and be passed along to daughter cells.

Some environments make errors more likely to arise and propagate. Such environments can include the presence of disruptive substances called carcinogens, repeated physical injury, heat, ionising radiation, or hypoxia. [28]

The errors which cause cancer are self-amplifying and compounding, for example:

- A mutation in the error-correcting machinery of a cell might cause that cell and its children to accumulate errors more rapidly.
- A further mutation in an oncogene might cause the cell to reproduce more rapidly and more frequently than its normal counterparts.
- A further mutation may cause loss of a tumour suppressor gene, disrupting the apoptosis signalling pathway and resulting in the cell becoming immortal.
- A further mutation in signalling machinery of the cell might send error-causing signals to nearby cells.

The transformation of normal cell into cancer is akin to a chain reaction caused by initial errors, which compound into more severe errors, each progressively allowing the cell to escape the controls that limit normal tissue growth. This rebellion-like scenario becomes an undesirable survival of the fittest, where the driving forces of evolution work against the body's design and enforcement of order. Once cancer has begun to develop, this on-going process, termed clonal evolution drives progression towards more invasive stages.

**Diagnosis**

Most cancers are initially recognized either because of the appearance of signs or symptoms or through screening. Neither of these leads to a definitive diagnosis, which requires the examination of a tissue sample by a pathologist. People with suspected cancer are investigated with medical tests. These commonly include blood tests, X-rays, CT scans and endoscopy.
Classification

Cancers are classified by the type of cell that the tumour cells resemble and therefore presumed to be the origin of the tumour. These types include:

- Carcinoma: Cancers derived from epithelial cells. This group includes many of the most common cancers, particularly in the aged, and include nearly all those developing in the breast, prostate, lung, pancreas, and colon.
- Sarcoma: Cancers arising from connective tissue (i.e. bone, cartilage, fat, nerve), each of which develop from cells originating in mesenchymal cells outside the bone marrow.
- Lymphoma and leukemia: These two classes of cancer arise from hematopoietic (blood-forming) cells that leave the marrow and tend to mature in the lymph nodes and blood, respectively. Leukemia is the most common type of cancer in children accounting for about 30%.
- Germ cell tumor: Cancers derived from pluripotent cells, most often presenting in the testicle or the ovary (seminoma and dysgerminoma, respectively).
- Blastoma: Cancers derived from immature "precursor" cells or embryonic tissue. Blastomas are more common in children than in older adults.

Cancers are usually named using -carcinoma, -sarcoma or -blastoma as a suffix, with the Latin or Greek word for the organ or tissue of origin as the root. For example, cancers of the liver parenchyma arising from malignant epithelial cells is called hepatocarcinoma, while a malignancy arising from primitive liver precursor cells is called a hepatoblastoma, and a cancer arising from fat cells is called a liposarcoma. For some common cancers, the English organ name is used. For example, the most common type of breast cancer is called ductal carcinoma of the breast. Here, the adjective ductal, refers to the appearance of the cancer under the microscope, which suggests that it has originated in the milk ducts.

Benign tumours (which are not cancers) are named using -oma as a suffix with the organ name as the root. For example, a benign tumor of smooth muscle cells is called a leiomyoma (the common name of this frequently occurring benign tumor in the uterus is fibroid). Confusingly, some types of cancer use the -noma suffix, examples including melanoma and seminoma. Some types of cancer are named for the size and shape of the cells under a microscope, such as giant cell carcinoma, spindle cell carcinoma, and small cell carcinoma.
Figure 14 Types of Cancer

Pathology

The tissue diagnosis given by the pathologist indicates the type of cell that is proliferating, its histological grade, genetic abnormalities, and other features of the tumour. Together, this information is useful to evaluate the prognosis of the patient and to choose the best treatment. Cytogenetics and immuno-histochemistry are other types of testing that the pathologist may perform on the tissue specimen. These tests may provide information about the molecular changes (such as mutations, fusion genes, and numerical chromosome changes) that has happened in the cancer cells, and may thus also indicate the future behaviour of the cancer (prognosis) and best treatment.
**Prevention**

Cancer prevention is defined as active measures to decrease the risk of cancer \[29\]. The vast majority of cancer cases are due to environmental risk factors, and many, but not all, of these environmental factors are controllable lifestyle choices. Thus, cancer is considered a largely preventable disease. \[30\] Greater than 30% of cancer deaths could be prevented by avoiding risk factors including: tobacco, overweight / obesity, an insufficient diet, physical inactivity, alcohol, sexually transmitted infections, and air pollution. \[31\] Not all environmental causes are controllable, such as naturally occurring background radiation, and other cases of cancer are caused through hereditary genetic disorders, and thus it is not possible to prevent all cases of cancer.

**Diet**

While many dietary recommendations have been proposed to reduce the risk of cancer, few have significant supporting scientific evidence. \[32\] The primary dietary factors that increase risk are obesity and alcohol consumption; with a diet low in fruits and vegetables and high in red meat being implicated but not confirmed. \[33\] Consumption of coffee is associated with a reduced risk of liver cancer. \[31\] Studies have linked consumption of red or processed meat to an increased risk of breast cancer, colon cancer, and pancreatic cancer, a phenomenon which could be due to the presence of carcinogens in meats cooked at high temperatures. Dietary recommendations for cancer prevention typically include an emphasis on vegetables, fruit, whole grains, and fish, and an avoidance of red meat, animal fats and refined carbohydrates. However, these recommendations are based on relatively limited evidence.

**Medication**

The concept that medications can be used to prevent cancer is attractive, and evidence supports their use in a few defined circumstances. In the general population NSAIDs reduce the risk of colorectal cancer however due to the cardiovascular and gastrointestinal side effects they cause overall harm when used for prevention. \[34\] Aspirin has been found to reduce the risk of death from cancer by about 7%. \[35\] COX-2 inhibitor may decrease the rate of polyp formation in people with familial adenomatous polyposis however are associated with the same adverse effects as NSAIDs. Daily use of tamoxifen or raloxifene has been demonstrated to reduce the risk of developing breast cancer in high-risk women. The benefit v/s harm for 5-alpha reductase inhibitor such as finasteride is not clear.
Vitamins have not been found to be effective at preventing cancer, although low blood levels of vitamin D are correlated with increased cancer risk. Whether this relationship is causal and vitamin D supplementation is protective is not determined. Beta-carotene supplementation has been found to increase lung cancer rates in those who are high risk. Folic acid supplementation has not been found effective in preventing colon cancer and may increase colon polyps.

**Vaccination**

Vaccines have been developed that prevent some infection by some viruses. Human papilloma virus vaccine (Gardasil and Cervarix) decreases the risk of developing cervical cancer. The hepatitis B vaccine prevents infection with hepatitis B virus and thus decreases the risk of liver.

**Screening**

Unlike diagnosis efforts prompted by symptoms and medical signs, cancer screening involves efforts to detect cancer after it has formed, but before any noticeable symptoms appear. This may involve physical examination, blood or urine tests, or medical imaging. Cancer screening is currently not possible for many types of cancers, and even when tests are available, they may not be recommended for everyone. Universal screening or mass screening involves screening everyone. Selective screening identifies people who are known to be at higher risk of developing cancer, such as people with a family history of cancer. Several factors are considered to determine whether the benefits of screening outweigh the risks and the costs of screening. These factors include:

- The likelihood of the test correctly identifying cancer.
- The likelihood of cancer being present: Screening is not normally useful for rare cancers.
- Possible harms from follow-up procedures.
- Whether suitable treatment is available.
- Whether early detection improves treatment outcomes.
- Whether the cancer will ever need treatment.
• Whether the test is acceptable to the people: If a screening test is too burdensome (for example, being extremely painful), then people will refuse to participate.
• Cost of the test.

Recommendations
The U.S. Preventive Services Task Force (USPSTF) strongly recommends cervical cancer screening in women who are sexually active and have a cervix at least until the age of 65. They recommend that Americans be screened for colorectal cancer via faecal occult blood testing, sigmoidoscopy, or colonoscopy starting at age 50 until age 75. There is insufficient evidence to recommend for or against screening for skin cancer, oral cancer, lung cancer or prostate cancer in men under 75. Routine screening is not recommended for bladder cancer, testicular cancer, ovarian cancer, pancreatic cancer or prostate cancer.

The USPSTF recommends mammography for breast cancer screening every two years for those 50–74 years old. However, they do not recommend either breast self-examination or clinical breast examination. A 2011 Cochrane review came to slightly different conclusions with respect to breast cancer screening stating that routine mammography may do more harm than good. Japan screens for gastric cancer using photofluorography due to the high incidence there.

Genetic Testing

Genetic Testing

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cancer types</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1, BRCA2</td>
<td>Breast, ovarian, pancreatic</td>
</tr>
<tr>
<td>HNPCC, MLH1, MSH2, MSH6, PMS1, PMS2</td>
<td>Colon, uterine, small bowel, stomach, urinary tract</td>
</tr>
</tbody>
</table>

Genetic testing for individuals at high-risk of certain cancers is recommended. Carriers of these mutations may then undergo enhanced surveillance, chemoprevention, or preventative surgery to reduce their subsequent risk.
Management
Many management options for cancer exist with the primary ones including surgery, chemotherapy, radiation therapy, and palliative care. Which treatments are used depends upon the type, location and grade of the cancer as well as the person's health and wishes.

Palliative Care
Palliative care refers to treatment which attempts to make the patient feel better and may or may not be combined with an attempt to attack the cancer. Palliative care includes action to reduce the physical, emotional, spiritual, and psycho-social distress experienced by people with cancer. Unlike treatment that is aimed at directly killing cancer cells, the primary goal of palliative care is to improve the patient's quality of life.

![Diagram of cell cycle and cancer treatment](image)

**Figure-15** Treatment of Cancer

Patients at all stages of cancer treatment need some kind of palliative care to comfort them. In some cases, medical specialty professional organizations recommend that patients and physicians
respond to cancer only with palliative care and not with cancer-directed therapy. Those cases have the following characteristics:

1. Patient has low performance status, corresponding with limited ability to care for oneself.
2. Patient received no benefit from prior evidence-based treatments.
3. Patient is ineligible to participate in any appropriate clinical trial.
4. The physician sees no strong evidence that treatment would be effective.

Palliative care is often confused with hospice and therefore only involved when people approach end of life. Like hospice care, palliative care attempts to help the person cope with the immediate needs and to increase the person's comfort. Unlike hospice care, palliative care does not require people to stop treatment aimed at prolonging their lives or curing the cancer.

Multiple national medical guidelines recommend early palliative care for people whose cancer has produced distressing symptoms (pain, shortness of breath, fatigue, nausea) or who need help coping with their illness. In people who have metastatic disease when first diagnosed, oncologists should consider a palliative care consult immediately. Additionally, an oncologist should consider a palliative care consult in any patient they feel has a prognosis of less than 12 months even if continuing aggressive treatment.

**Surgery**

Surgery is the primary method of treatment of most isolated solid cancers and may play a role in palliation and prolongation of survival. It is typically an important part of making the definitive diagnosis and staging the tumour as biopsies are usually required. In localized cancer surgery typically attempts to remove the entire mass along with, in certain cases, the lymph nodes in the area. For some types of cancer this is all that is needed to eliminate the cancer.

**Chemotherapy**

Chemotherapy in addition to surgery has proven useful in a number of different cancer types including, breast cancer, colorectal cancer, pancreatic cancer, osteogenic sarcoma, testicular cancer, ovarian cancer, and certain lung cancers. The effectiveness of chemotherapy is often limited by toxicity to other tissues in the body.
Radiation

Radiation therapy involves the use of ionizing radiation in an attempt to either cure or improve the symptoms of cancer. It is used in about half of all cases and the radiation can be from either internal sources in the form of brachytherapy or external sources. Radiation is typically used in addition to surgery and or chemotherapy but for certain types of cancer such as early head and neck cancer may be used alone. For painful bone metastasis it has been found to be effective in about 70% of people.

Alternative Treatments

Complementary and alternative cancer treatments are a diverse group of health care systems, practices, and products that are not part of conventional medicine. "Complementary medicine" refers to methods and substances used along with conventional medicine, while "alternative medicine" refers to compounds used instead of conventional medicine. Most complementary and alternative medicines for cancer have not been rigorously studied or tested. Some alternative treatments have been investigated and shown to be ineffective but still continue to be marketed and promoted.

![Figure 16: Adverse Effects]

Figure-16 Adverse Effects
1.4 INTRODUCTION TO HERBAL MEDICINE

Herbal medicine is still the mainstay of about 75–80% of the world population, mainly in the developing countries, for primary health care because of better cultural acceptability, better compatibility with the human body and lesser side effects. However, the last few years have seen a major increase in their use in the developed world. India is sitting on a gold mine of well-recorded and well-practiced knowledge of traditional herbal medicine. But, unlike China, India has not been able to capitalize on this herbal wealth by promoting its use in the developed world despite their renewed interest in herbal medicines. This can be achieved by judicious product identification based on diseases found in the developed world for which no medicine or only palliative therapy is available; such herbal medicines will find speedy access into those countries.\(^{[38]}\)

The basic requirements for gaining entry into developed countries include:

i. Well-documented traditional use.

ii. Single plant medicines.

iii. Medicinal plants free from pesticides, heavy metals.

iv. Standardization based on chemical and activity profile.

v. Safety and stability.

However, mode of action studies in animals and efficacy in human will also be supportive. Such scientifically generated data will project herbal medicine in a proper perspective and help in sustained global market.

**Evidence of Herbal Medicine**

The traditional preparations comprise medicinal plants, minerals, organic matter etc. Herbal drugs constitute only those traditional medicines which primarily use medicinal plant preparations for therapy. The earliest recorded evidence of their use in Indian, Chinese, Egyptian, Greek, Roman and Syrian texts dates back to about 5000 years. The classical Indian texts include Rigveda, Atherveda, Charak Samhita and Sushruta Samhita.

**Choice of Herbal Medicine**

Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care. They have stood the test of time for their safety, efficacy. The chemical constituents present in them are a part of the physiological functions of living flora and
hence they are believed to have better compatibility with the human body. Ancient literature also mentions herbal medicines for age related diseases namely memory loss, osteoporosis, diabetic wounds, immune and liver disorders etc., for which no modern medicine or only palliative therapy is available. These drugs are made from renewable resources of raw materials by eco-friendly processes and will bring economic prosperity to the masses growing these raw materials.

**Herbal Medicine Scenario in India**

The turnover of herbal medicines in India as over-the-counter products, ethical and classical formulations and home remedies of Ayurveda, Unani and Siddha systems of medicine is about $1 billion with a meagre export of about $ 80 million. Psyllium seeds and husk, castor oil and opium extract alone account for 60% of the exports. 80% of the exports to developed countries are of crude drugs and not finished formulations leading to low revenue for the country. India is one of the 12 mega biodiversity centre having over 45,000 plant species. Its diversity is unmatched due to the presence of 16 different agro climatic zones, 10 vegetative zones and 15 biotic provinces. The country has 15,000–18,000 flowering plants, 23,000 fungi, 2500 algae, 1600 lichens, 1800 bryophytes and 30 million micro-organisms. [39]

**Role of WHO in Herbal Medicine**

Disease or ill health is brought about by an imbalance or disequilibrium of man in his total ecological system and not only by the causative agent and pathologic evolution (WHO). [40] In 1991 WHO developed guidelines for the assessment of herbal medicine, [41] and the same were ratified by the 6th International Conference of Drug Regulatory Authorities held at Ottawa in the same year. The salient features of WHO guidelines are:

i. Quality assessment: Crude plant material; Plant preparation; Finished product.

ii. Stability: Shelf life.

iii. Safety assessment: Documentation of safety based on experience or/and; Toxicology studies.

iv. Assessment of efficacy: Documented evidence of traditional use or/and; Activity determination (animals, human).
Safe and Unsafe Herbs

Some herbal medicines are considered to be comparatively safe. These include Feverfew (*Tanacetum parthenium*) used in the prophylaxis of migraine headaches, treatment of fever, menstrual problems, asthma, dermatitis and arthritis. Garlic (*Allium sativum*) is used in hyperlipoproteinemia and prevents arteriosclerosis. Other herbs are Ginkgo (*Ginkgo biloba*), saw palmetto (*Sereno arepens*), Asian ginseng (*Panax ginseng*), St. John’s Wort (*Hypericum perforatum*), Valerian (*Valeriana officinalis*) and Ginger (*Zingiber officinale*). They do possess some therapeutic value and are increasingly the subject of clinical trials in which their efficacy, tolerability and safety are being compared with allopathic medicines. Some herbs possess constituent with toxic potential. These include *Acorus calamus* (mutagenic and carcinogenic), *Anthoxanthum odoratum* (hepatotoxic), *Artemisia absinthium* (neurotoxic), *Callilepis laureola* (nephrotoxic and hepatotoxic), *Conium maculae* (teratogenic), *Croton tiglium* (purge and tumour promoting), *Stephania spp.* (CNS depressant and hepatotoxic), *Erythroxylum spp.* (Psychotism) and *Glycyrrhiza* (pseudoaldosteronism) considered carcinogenic, Borage (*Borago officinalis*), Comfery (*Symphy spp.*) and Life root (*Scenecio aureus*). Unfortunately, checking the label herbal package is not always sufficient to exclude the potential contaminations because some of the currently available preparations are contaminated intentionally or accidentally, or by potential herbal or non-herbal substance and sometimes with microorganisms. [42]

Fingerprinting / Chemoprofiling

Fingerprinting in essence is chemoprofiling while establishing a characteristic chemical pattern for a plant material or its cut or fraction of extract. It is important to understand that a plant extract consists of establishment of chemical compounds. These include the primary metabolites, secondary metabolites and inorganic metals. Primary metabolites are components of carbohydrates, proteins, lipids which are essential for the plant physiology. Secondary metabolites are metabolites which are not essential for plant physiology and are formed as by-products in the biochemical reactions. These include very interesting and useful compounds like alkaloids, flavonoids, coumarins, anthocyanin’s, etc. Many modern drugs have come from secondary metabolites like morphine from opium, serpentine from rauwolfia, vinblastine and vincristine from *Vinca rosea*. In fact this is the point of divergence for modern system of medicine and classical system of medicine like ayurveda with the former laying emphasis on compounds. However coming to the main line, one can utilize these secondary metabolites for the identification of plant
material as our knowledge of chemistry advanced sufficiently and through sophisticated techniques we can measure these compounds qualitatively and quantitatively. But the catch lies here for two factors first, if in a plant material we can measure the presence of unique secondary metabolites it is not sufficient to decide certainly that the plant material is of the desired quality. Second important factor is that there are no data for characterizing compounds even for phytochemical studied materials. The herbal drug industry is looking into number of these characterizing compounds obtained from specific plant materials.⁴³

**MULTIDISCIPLINARY APPROACHES:**

![Pathway from Plant to Pure Bioactive Constituents](image)

**Figure-17** Pathway from Plant to Pure Bioactive Constituents

**STANDARDISATION AND QUALITY EVALUATION PARAMETERS OF HERBAL DRUGS:**

For safe and effective use of herbal drugs, consistency in composition and biological activity are essential. However, herbal drugs frequently fail to meet the standard due to some problems such as

- Difficulties in identification of plants.
- Genetic variability.
- Variations in growing conditions.
- Diversity in harvesting procedures and processing of extracts.
- Lack of knowledge about active pharmacological principles.
Batch-to-batch consistency can be ensured by performing standardization of herbal products with the help of chromatographic techniques and marker compounds. The lack of standardization of herbal drugs would be a serious problem for a researcher as he would not be able to rely on commercially available herbal products for his research study. The standardization and quality evaluation parameter of herbal drugs is as follows:

**AUTHENTICATION**
- RADIO ACTIVE CONTAMINANTS
- MICROBIAL COUNT
- HEAVY METALS
- PESTICIDE RESIDUE
- MARKER COMPONENT
- CHROMATOGRAPHIC PROFILE

**FOREIGN MATTER**
- ORGANOLEPTIC EVALUATION
- MACROSCOPY AND MICROSCOPY
- VOLATILE MATTER
- ASH VALUE
- EXTRACTIVE VALUE

**Figure-18**

Herbal drugs standardization can be done by performing screening. Screening implies the evaluation of multiple samples in a ritualized fashion using a standardized single technique or tests, which is proportionally more expensive and tedious.

**Screening of herbal drugs for biological activity include**[^44]

- Primary pharmacological screening
- Secondary and tertiary acute pharmacological/toxicological evaluations
- Chronic pharmacological/toxicological evaluations
- Product formulations
- Clinical trials
- Release in system for therapeutic utilization

[^44]: [44]
SCREENING APPROACHES

Three types of screening approaches define herbal drugs, via

- Primary
- Secondary
- Tertiary

PRIMARY SCREENING

Past Approach
Phytochemical and chemotherapeutic screening remains the dominant trends for screening programs.

Present Approach
The pharmacological screening can be divided into four styles

Single Technique-Single Goal Screening
This utilizes a single technique aiming at a single goal as the activity of natural product.

Screening Using a Battery of Specific Procedures
This utilizes multiple specific tests to define pharmacological activity of a crude drug.

Single Technique Multiple Goals Screening
This utilizes multiple observations-single techniques to search for virtually any and all pharmacological activity in single crude drugs.

Combination of Specific and Multipurpose Procedures
This includes various specific and multipurpose procedures.

SECONDARY SCREENING
Primary screening is always conducted using only minimum amount of carefully authenticated crude drug. If promising activity has been found during primary screening then a sizeable quantity of authenticated plant material is acquired and secondary evaluations organized. Secondary testing is confirmed in another species of laboratory animals, the activity noted in primary screen should consist of drug or drug interaction experiments.
TERTIARY SCREENING

Tertiary screening is expensive and time consuming. The course taken for a single drug depends on data accumulated during primary and secondary phases of evaluation and open for further research. All these methods account for a single chemical entity or a group of chemical compounds, but in many plants the activity may be attributed to different types of compounds that act synergistically to show the desired biological activity. Therefore, standardization by chemical methods, although used widely, may not prove to be a complete way of standardization and further need biological standardization.

BIOLOGICAL ASSAYS

Biological assays ensures consistent clinical efficacy of herbal product from batch to batch. The analytical methods including chromatographic evaluation are sometimes ineffective as they are usually insensitive to the chemical complexities found in crude botanical extracts. The biological potency of the herbal drug is due to not one but a mixture of bioactive plant constituents. The relative properties of a single bioactive compound can vary from batch to batch while the biological activity remains within the desirable limits. Thus, it is desirable to incorporate bioassay as an additional method of standardization, which in turn becomes an effective quality control method.

Bioassays are broadly classified as:

- General Screening Bioassay
- Specific Bioassay
- Primary Screening Bioassay

General Screening Bioassay

These are non-selective and indicate biological activity of the herbal drugs.

Primary Screening Bioassay

This helps in identification of bioactive compounds.

Specific Bioassay

It provides specific bioactivity and a large number of herbal drugs have been standardized by using this method.
**REVERSE PHARMACOLOGY**

Reverse pharmacology is defined as the science of integrating documented clinical experiences and experimental observations into leads by transdisciplinary exploratory studies and further developing these into drug candidates or formulations through robust preclinical and clinical research. The traditional knowledge inspired reverse pharmacology described here relates to reversing the routine ‘laboratory to clinic’ progress of discovery pipeline to ‘clinics to laboratories’. In this progress safety remains the most important starting point and the efficacy becomes a matter of validation. Sir Ram Nath Chopra and Gananath Sen laid the foundation of reverse pharmacology of medicinal plants by pursuing clinically documented effects of ayurvedic drugs. *Rauwolfia serpentine* Benth, was a major discovery via this approach. Sen and Bose in 1931 convincingly demonstrated the antihypertensive and tranquillizing effects of the plant and also observed unique side effects such as depression, extra pyramidal syndrome, gynacomastia and peptic ulcer.

Clinical events or phenomenon previously not reported and following the administration of a known or new drug, can provide valuable insights for drug development. Medicinal plants and natural products derived, from there have provided many such serendipitous bedside observations. Historically, several such clinical hits were not often pursued quickly and rigorously by the drug discovery teams. Similarly, research in genomics, proteomics and metabolomics has stimulated discovery of many new entities, which are yet to be pursued for their drug-like activities. A new trans-disciplinary endeavour called reverse pharmacology has recently emerged and addresses both these needs. Reverse Pharmacology (RP), designed as an academic discipline helps to reduce three major bottle necks of costs, time and toxicity. RP can be perceived to comprise of three phases. First, the experimental phase that includes robust documentation of clinical observations of the biodynamic effects of standardized ayurvedic drugs by meticulous record keeping. Second, the exploratory studies for tolerability, drug-interactions, dose-range finding in ambulant patients of defined subsets of the disease and paraclinical studies in relevant *invitro* and *in vivo* models to evaluate the target-activity. Third phase includes experimental studies, basic and clinical, at several levels of biological organization, to identify and validate the reverse pharmacological correlates of ayurvedic drug safety and efficacy. The scope of reverse pharmacology is to understand the mechanisms of action at multiple levels of biology and to optimize safety, efficacy and acceptability of the leads in natural products based on relevant science. In this approach, the
candidate travels a reverse path from ‘clinics to laboratory’ rather than classical ‘laboratory to clinics’. [50]

The conventional approach is seeking out new chemical drugs, which involves identifying the new molecules, testing their efficacy on laboratory animals, and then moving to humans. Chemical drug discovery around the world had focused on moving drugs from molecules to mice and then to men. Reverse pharmacology is the alternative and the most suitable approach for efficient discovery (rediscovery) of herbal drugs with very few bottle necks. In reverse pharmacology we are going the other way—from men to mice to men. Traditional herbal medicine has long been used in clinical practice. Reverse pharmacology is aimed at validating such herbal drugs through modern scientific methods.

Scope
The scope of reverse pharmacology is to understand the mechanisms of action at multiple levels of biological organization and to optimize safety, efficacy and acceptability of the leads in natural products, based on relevant science. [51]

Figure-19 Conventional Approach v/s Reverse Pharmacological Approach
Screening Methods

Analgesic Activity

1. Chemically induced nociception (A) Writhing tests. \textsuperscript{[52]}
2. Randall-Selitto test \textsuperscript{[53]}
3. Intra-arterial Bradykinin test \textsuperscript{[54]}
4. Formalin test in rats \textsuperscript{[55]}
5. Electrical stimulation methods (A) electrical stimulation of the tail \textsuperscript{[56]}
6. Flinch-jump testing mice \textsuperscript{[57]}
7. Tooth pup stimulation in rabbits \textsuperscript{[58]}
8. Mechanical stimulation methods (A) Heffner’s tail-clip tests in mice \textsuperscript{[59]}
9. Thermal stimulation methods (A) radiant heat method (Tail-flick test) \textsuperscript{[60]}
10. Tail immersion test \textsuperscript{[61]}
11. Hot plate method \textsuperscript{[62]}
12. Differentiation of peripheral and central effects of Analgesics \textsuperscript{[63]}

Anti-inflammatory activity

1. Methods for testing acute and sub-acute inflammation \textsuperscript{[64]}
2. Ultraviolet erythema in Guinea pigs \textsuperscript{[65]}
3. Croton-oil ear oedema in rats and mice \textsuperscript{[66]}
4. Oxazolone-induced ear oedema in mice \textsuperscript{[67]}
5. Carrageenan-induced pleurisy in rats \textsuperscript{[68]}
6. Granuloma pouch in rats \textsuperscript{[69]}
7. Methods for testing the proliferative phase of inflammation (A) cotton wool granuloma \textsuperscript{[70]}
8. Sponge implantation in rats \textsuperscript{[71]}
9. Glass rod granuloma in rats \textsuperscript{[72]}
10. Adjuvant-induced arthritis in rats \textsuperscript{[73]}

Anti-ulcer activity

1. Anti-secretory activity.
   a. Gosh and Schild perfused rat stomach preparation \textsuperscript{[74]}
   b. Gastric and Heidenhain pouch in the dog \textsuperscript{[75]}
   c. Isolated whole stomach preparation of rat \textsuperscript{[76]}
   d. Chronic gastric in dogs \textsuperscript{[77]}
2. Gastric anti-ulcer activity (A) pylorus ligated (shay) rats \[78\]

3. Stress ulcers (A) restraint ulcers in rats \[79\]

4. Water immersion-induced restraint ulcers \[80\]

5. Cold and restraint ulcers \[81\]

6. Gastric mucosal damage induced by NSAID’s in rats.
   a. Aspirin \[82\]
   b. Phenyl Butazone \[83\]
   c. Indomethacin \[84\]

7. Induced solitary chronic gastric ulcers \[85\]

8. Acetic acid induced “kissing” gastric ulcers in rats \[86\]

9. Gastric injury in rats \[87\]

10. Histamine induced gastric ulcers in Guinea pigs \[88\]

11. Duodenal Anti-ulcer activity \[89\]

12. Cysteamine-induced duodenal ulcers in rats \[90\]

13. Dulcerozine induced duodenal ulcers in rats \[91\]

14. Indomethacin + Histamine induced duodenal ulcers in rats \[92\]

15. Other models of duodenal ulceration (A) gastric cytoprotective activity \[93\]

**Central Nervous System**

1. Observational assessment \[94\]

2. Studies related to safety pharmacology.
   a. Sedative or stimulatory activity \[95\]
   b. Method of intermittent observations \[96\]
   c. Open field test \[97\]
   d. Hole-Board test in mice \[98\]

3. Tests for muscle co-ordination.
   a. Inclined plane test in mice \[99\]
   b. Chimney test in mice \[100\]
   c. Grip strength in mice \[101\]
   d. Rota rod test in mice \[102\]
**Cardio tonic Activity**
1. The frog method \[^{103}\]
2. The pigeon method \[^{104}\]
3. Hatcher’s cardio toxicity in cat \[^{105}\]
4. Experimentally induced cardiac insufficiency in guinea pigs \[^{106}\]
5. Cardiomyopathy in Syrian Hamsters \[^{107}\]
6. Isolated cat papillary muscle \[^{108}\]
7. Loss of potassium from isolated guinea pig heart \[^{109}\]

**Hepatoprotective activity**
1. Hepatitis in long Evans cinnamon rats \[^{110}\]
2. Allyl alcohol-Induced liver damage in rats \[^{111}\]
3. Carbontetra chloride (CCl\(_4\)) induced liver damage in rats \[^{112}\]
4. Bile duct induced fibrosis of liver in rats \[^{113}\]
5. Galactoseamine induced necrosis of liver \[^{114}\]
6. Thiocetamide induced Hepatotoxicity in rats \[^{115}\]
7. Paracetemol-induced liver damage in rats \[^{116}\]
8. Rifampicin + Isoniazid induced Hepatotoxicity in rats \[^{117}\]

**Cytotoxic activity**
1. Short term cytotoxic activity \[^{118}\]
2. Long term cytotoxic activity \[^{119}\]