# Chapter 2

## Chapter 2: Review of literature

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Name of the Sub-Title</th>
<th>Page No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.</td>
<td>Review of literature</td>
<td>14-142</td>
</tr>
<tr>
<td>2.1</td>
<td>Literature Review of <em>Terminalia pallida</em> Brandis.</td>
<td>15</td>
</tr>
<tr>
<td>2.2</td>
<td>Literature Review of <em>Boswellia ovalifoliolata</em></td>
<td>19</td>
</tr>
<tr>
<td>2.3</td>
<td>Antimicrobials</td>
<td>23</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Methods to assay antimicrobial activity</td>
<td>23</td>
</tr>
<tr>
<td>2.3.2</td>
<td>Microbes</td>
<td>26</td>
</tr>
<tr>
<td>2.4</td>
<td>Antioxidant</td>
<td>31</td>
</tr>
<tr>
<td>2.4.1</td>
<td>Endogenous antioxidants</td>
<td>33</td>
</tr>
<tr>
<td>2.4.2</td>
<td>Exogenous antioxidants</td>
<td>35</td>
</tr>
<tr>
<td>2.5</td>
<td>Inflammation</td>
<td>36</td>
</tr>
<tr>
<td>2.5.1</td>
<td>Types of inflammation</td>
<td>38</td>
</tr>
<tr>
<td>2.5.2</td>
<td><em>In vivo</em> models of anti-inflammatory activity</td>
<td>51</td>
</tr>
<tr>
<td>2.6</td>
<td>Analgesics</td>
<td>63</td>
</tr>
<tr>
<td>2.6.1</td>
<td>Animal models to screen analgesic activity</td>
<td>76</td>
</tr>
<tr>
<td>2.7</td>
<td>Hepatoprotectives</td>
<td>112</td>
</tr>
<tr>
<td>2.7.1</td>
<td><em>In vivo</em> Models for hepatoprotectives</td>
<td>112</td>
</tr>
</tbody>
</table>
Chapter 2

2. Review of literature

2.1 Literature Review of *Terminalia pallida* Brandis.

Table 1: Scientific Classification of *Terminalia pallida* Brandis

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Myrtales</td>
</tr>
<tr>
<td>Family</td>
<td>Combretaceae</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Terminalia</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>Terminalia pallida</em></td>
</tr>
</tbody>
</table>

Botanical Name: *Terminalia pallida* Brandis.

Synonym: *Terminalia alata, Terminalia bellerica*

Family: Combretaceae

Common Names:

English : White gallnu

Distribution: It is spread in the tropical and subtropical regions of the world. It occurs in the stony hilly areas of dry deciduous forests of Chittoor, Cuddapah and Kurnool, Tirumala Hills, India, Nepal, Pakistan, Srilanka, China, Malaydia, Myanmar and Thailand.
Figure 1: *Terminalia pallida* Brandis

**Plant Description:**

*Terminalia pallida* is herb from the family Combretaceae. *Terminalia pallida* is semi-evergreen tree group. Leaf fall and flushing events occur during premonsoon season; leaf flushing extends into the monsoon season. The flowers are bisexual and obligately outcrossed and enforced by self-incompatibility. Flowering occurs after dry season and before monsoon season. Protogyny promotes outcrossing, but it is weak. Individual trees flower for a brief period of three weeks with massive floral display. The flowers propose both nectar and pollen for the foragers; the nectar gives essential amino acids along with some non-essential amino acids. The plant is entomophilous, and cross-pollination is effected mainly by large bees, wasps and butterflies. Fruits fall to the ground when mature and dry in presence of wind. The fallen fruits are
scatter by rain water and the seeds germinate and establish seedlings depending on the soil status\textsuperscript{15,16}.

Leaves : Alternate, sub-opposite, ovate, petioles hort with glands beneath.

Flowers : Small, green, in spikes. Calyx campanulate.

Fruit : Obovoid, very faintly 5-ridged when dry, glabrous, and indehiscent.

**Traditional Uses:**

*Terminalia pallida* (TP) Brandis is one of the oldest medicinal herb of India, is an ingredient of Indian Ayurvedic drug 'triphala' used for the treatment of digestion and liver disorders. In Indian traditional system of medicine, the fruits are also used in the treatment of hepatic disorders and treatment of diabetes by tribal people\textsuperscript{10}.

The leaf is used for treating skin blisters and skin diseases, whereas the stem bark is used as a diuretic and for swellings. The fruit is used as an anti-pyretic, purgative, for diarrhoea, peptic ulcers, diabetes, venereal diseases, cough, cold, dysentery, fissures, cracks and in tanning and dyeing. Bark powder is applied externally and can be taken internally as anti-inflammatory agent. Fruit powder is given orally to cure peptic ulcer fissure and to clear harshness of voice. \textsuperscript{17-23}. 
Reported biological activities:

- Antioxidant activity and hepatoprotective potential of *Terminalia pallida* was reported by Palani S. et.al.\(^\textsuperscript{10}\)

- Analgesic and antipyretic activity of *Terminalia pallida* stem was reported by Hamed A.S.et.al.\(^\textsuperscript{17}\)

- Antidiabetic activity of *Terminalia pallida* in alloxan induced diabetic rats was reported by Kameswara R.B. et.al.\(^\textsuperscript{18}\)

- Antiartritic activity of *Terminalia pallida* was reported by Hamed A.S.et.al.\(^\textsuperscript{19}\).

- Antiatherogenic and antihyperlipidemic activities of *Terminalia pallida* Linn. fruits in high fat diet-induced hyperlipidemic rats was reported by Sampathkuar M.T.et.al.\(^\textsuperscript{20}\)

- Anti-ulcer activity of ethanol extract of *Terminalia pallida* Brandis in Swiss albino rats was reported by Gupta M. et.al.\(^\textsuperscript{21}\)

- Phytochemical evaluation and antimicrobial studies on *Terminalia pallida* brandis (combretaceae) – a rare and endemic medicinal plant was reported by Kummari R. et.al.\(^\textsuperscript{22}\)
Phytoconstituents

The plant has many important phytoconstituents like Gallo-tannic acid, bellericanin, gallic acid, ellagic acid, termilignan, thanni lignin, flavones and anolignan B, Tannins, ethyl gallate, ellagic acid, gallonyl glucose and chebulaginic acid, phenyllembelin, beta-sitosterol, mannitol, rhamnose and glucose\textsuperscript{17-23}.

2.2 Literature Review of \textit{Boswellia ovalifoliolata}

\textbf{Botanical Name:} \textit{Boswellia ovalifoliolata}

\textbf{Table 2: Scientific Classification of \textit{Boswellia ovalifoliolata}}

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phylum</td>
<td>Magonoliophyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Order</td>
<td>Sapindales</td>
</tr>
<tr>
<td>Family</td>
<td>Burseraceae</td>
</tr>
<tr>
<td>Genus</td>
<td>\textit{Boswellia}</td>
</tr>
<tr>
<td>Species</td>
<td>\textit{Boswellia ovalifoliolata}</td>
</tr>
</tbody>
</table>

\textbf{Synonym:} \textit{Boswellia carterii, Boswellia dalziellii, Boswellia frereana}

\textbf{Family:} Burseraceae

\textbf{Common Names:}

Telugu: \textit{Guggilam, konda sambrani, adavi sambrani, sambrani}
**Distribution:**

The herb *boswellia ovalifoliolata* is scattered in the dry regions of Arabia, tropical Africa and India. In Arabia, it is mainly limited to Oman, Yemen and Socotra\textsuperscript{24}. In Africa, it is circulated in Sudan, Somalia, Ethiopia, Eritrea, Tanzania, Kenya, Madagascar and some other countries. In India, it is found in a few regions such as Rajasthan, southeast Punjab, Danwara and Madras.\textsuperscript{24}

![Boswellia ovalifoliolata](image)

**Figure 2: Boswellia ovalifoliolata**

**Plant Description:**

*Boswellia ovalifoliolata* (Burseraceae) is a narrow endemic and endangered deciduous herb species. Its blooming, fruiting and seed spreading actions happen during the dry season. The flowers are tiny, bisexual, gently odoriferous and actinomorphic; softly protandrous but strictly self-incompatible. The plant is known its medicinal uses. The fresh leaf juice used to stop throat ulcer. Decoction of the stem 10-25 ml
Insects and sunbirds pollinate the flowers. Both bud and flower feed by a weevil, bloom and fruit feed by the Palm Squirrel have been responsible for the achievement of sexual reproduction. The Garden Lizard offers as a predator of pollinating insects, mainly bees and wasps and thus influencing pollination. Adult fruits dehisce and spread their lightweight seeds with the support of wind. Seed germination occurs next to rainfall but further growth relies on soil, water and nutritional status. The success rate of seedling employment is highly restricted, and it could be depend on nutrient-poor soil and water stress ensuing from dry spells at the rainy season.\textsuperscript{24-27}

Leaves: Imparipinnate, alternate or crowded at the ends of branches; leaflets sessile, ovate-oblong, unequal at base, glabrous beneath, veins reddish.

Flowers: Small, bisexual, mildly odoriferous and actinomorphic, weakly protandrous but strictly self-incompatible.

Seed: Dispersal events occur in a leafless state during the dry season.

**Traditional Uses:**

The leaves, stem bark and gum resin are highly medicated and are traditionally used in the management of ulcers, dysentery, inflammation, arthritis, obesity, and diabetes. It has been used for cancer, liver disorders, acute asthma symptoms.\textsuperscript{24-27}. 
Reported biological activities:

- Antihyperlipidemic activity of *Boswellia ovalifoliolata* Bal.Henry was reported in atherogenic diet induced rats by Geetha K. et.al.\(^{28}\)

- *In-vitro* Antioxidant studies on ethanolic extracts of *Boswellia ovalifoliolata* and *Saccharum spontaneum* by DPPH, Nitric oxide and Lipid peroxidation methods was reported by Geetha K. et.al.\(^{29}\)

- Anti-inflammatory Activity of *Boswellia Ovalifoliolata* was reported in leaves by Selvan A.T. et.al.\(^{30}\)

- Anti-Microbial Activity was reported in Endamic Plant *Boswellia Ovalifoliolata* Leaves by Vasantha K.P. et.al.\(^{31}\)

- Antimicrobial activity of *Boswellia ovalifoliolata* against *xanthomonas – citri* and *salmonella typhynurium* was reported by Anitha G. et.al.\(^{32}\)

- Anti-ulcer activity of methanolic extract was reported for the leaves of *Boswellia ovalifoliolata* bal & henry by Dhanalakshmi M. et.al.\(^{33}\)

- *In vivo* analgesic activity of petroleum ether extract of *Boswellia ovalifoliolata Linn.* leaves was reported by Deepak B.K. et.al.\(^{34}\)
Phytoconstituents

Flavonoids like ovalifoliolatin A, ovalifoliolatin B, anthocyanins like leucoanthocyanins, cyaniding, phenols like cinamic acid, galic acid etc, lipids like phosphatidyl serine, indoles, steroids, amino acid like aspartic acid, saponins, tannins are reported into the literature.24-36

2.3 Antimicrobial study37-41

The main origin of many diseases is microbial infection. Air, water, food, sex are the vectors for infection. Antimicrobials are the agents that either terminate microbes or resist their growth. Antimicrobials can be categorised as per the microbe on which it acts. Varieties of antimicrobials are available according to its targeted microbe eg. if the microbe is bacteria antimicrobial agent termed as antibacterial, for fungus it termed as antifungal, etc. Antimicrobial activities found in the literature have been examined with varied sets of methodologies, degrees of sensitivity, amount of test compounds and microbial strains which are often difficult to compare.

2.3.1 Methods to assay antimicrobial activity

Agar absorption assay

1. Permit 20 ml nutrient agar plates to dry in 37°C incubator for 30 min.
2. Mandatory quantity of sample, standard or control then pipette over the plane of agar and spread using sterile glass spreader.

3. Plates then keep at upright position at room temperature for 30 min.

4. Spread chosen bacteria over the treated agar plate.

5. Incubate the glass plates overnight at 37°C and count the colonies formed next day.

**Agar Dilution assay**

1. Superimpose 5 ml agar containing sample, standard or control with and without 0.02% Tween 80 on 15 ml molden nutrient agar plates.

2. Control plates, one with agar alone and other with agar plus 0.02% Tween 80 should be used.

3. Streak solo colony of each bacterium on the face of the agar and incubate at 37°C overnight.

4. Bacterial broth can be denoted on the scale of 0 (No growth) to 4 (growth of control).

**Disc diffusion Assay**

1. Take dried (at 37°C for at least 30 min) nutrient agar plates.
2. Broaden overnight culture of bacteria (0.5ml) over the plane of agar plate by using sterile glass spreader and incubated at 37˚C for 30 min.

3. Add mandatory dose of sample, standard or control on sterile 6mm vacant antimicrobial susceptibility discs.

4. Keep the sample discs onto the incubated surface of agar plate (maximum of 5 discs per plate).

5. Incubate the glass plates overnight at 37˚C and count up the colonies produced next day.

**Well diffusion assay**

1. Inoculate 19 ml of molten nutrient agar with 0.5 ml of overnight culture.

2. The inoculated agar is then poured into sterile petriplates and then allows setting.

3. Then with the help of borer cut wells into plates.

4. Finally mandatory dose of sample, standard or control added into the wells.

5. Incubate the glass plates overnight at 37˚C and measure the zone of inhibition next day.
**Broath dilution assay**

1. The sample can be emulsified by adding 10 µl of 10% aqueous solution of Tween 80 into 90 µl of sample (1/10 volume). Then it requires vortexing.

2. Then put in nutrient broth in 10-20 µl aliquots with vortexing to make the final volume 1.5 ml.

3. An overnight bacterial culture is then supplemented to each tube.

4. Three tubes, 0.02% nutrient broth, sample containing 0.02% nutrient broth and only nutrient broth were prepared.

5. Mix and incubate the test tube at 37˚C for 12 hr.

6. Dilute each suspension tenfold serially with sterile nutrient broth.

7. Place 500µl of each concentration onto nutrient agar plate aseptically.

8. Incubate the glass plates overnight at 37˚C and count the colonies formed next day.

**2.3.2 Microbes**
Streptococcus pneumoniae 42

Streptococcus pneumoniae bacteria are lancet-shaped, gram-positive, facultative anaerobic in nature. They are naturally observed in pairs (diplococci) but may also found singularly or in short chains. Some pneumococci are encapsulated, their surfaces self-possessed of complex polysaccharides. Encapsulated organisms are pathogenic for humans and experimental animals. 90 serotypes have been identified, based on their reaction with type-specific antisera. Most of the S. pneumoniae serotypes have been shown as source of serious disease, but only a few serotypes produce the majority of pneumococcal infections. The key clinical syndromes of pneumococcal disease are pneumonia, bacteremia, and meningitis. Pneumococci are a universal cause of acute otitis media.

Staphylococcus aureus 43,44

The staphylococci are non-motile, non-spore forming facultative anaerobes that grow by aerobic respiration or by fermentation. Staphylococcus aureus is a gram positive microorganism and leading cause of hospital-acquired infections. It is the primary cause of lower respiratory tract infections and surgical site infections and the second leading cause of nosocomial bacteremia, pneumonia, and cardiovascular infections. Infections with S. aureus are especially difficult to treat because of evolved resistance to antimicrobial drugs.
**Bacillus cereus**

*Bacillus cereus* is a Gram-positive, spore-forming microorganism capable of causing foodborne disease. At present three enterotoxins, able to cause the diarrheal syndrome, have been described: hemolysin BL (HBL), nonhemolytic enterotoxin (NHE) and cytotoxin K. HBL and NHE are three-component proteins, whereas cytotoxin K is a single protein toxin.

**Bacillus pumilis**

The Gram-positive, aerobic, rod-shaped endospore-forming bacteria of the Genus *Bacillus* are the most widely represented organisms in the soil. Due to their ability to form spores and withstand a range of variable environmental conditions, *Bacillus* spp. adapt easily to diverse habitats.

**Escherichia coli**

*Escherichia coli* (*E. coli*) are bacteria which live in the intestines of healthy people and animals, primarily cattle. Most of the strains of *Enterobacter aerogenes* are harmless. *E. coli* 0157:H7 is a specific strain of *E. coli* that causes illness. It was first recognized as a cause of illness during an outbreak of hemorrhagic colitis (severe bloody diarrhea).
**Enterobacter aerogenes**

*Enterobacter* spp. are rising as important pathogens for a wide variety of nosocomial infections which includes pneumonia, urinary tract infections, meningitis, wound infections, and infections connected to intravascular and prosthetic devices. *E. aerogenes* can cause primary bacteremia in pediatric patients, mediastinitis subsequent cardiac surgery, and crepitant cellulitis. *Enterobacter* bacteremia has been reported and the mortality rate for *Enterobacter* bacteremia was 29% in one study.

**Pseudomonas aeruginosa**

Pseudomonas aeruginosa is a chief cause of nosocomial infections. Acknowledged for the reason of serious wound and surgical infections, but a lot regarded as a secondary invader rather than a cause of primary infection in healthy tissues, *Pseudomonas aeruginosa* has now clearly emerged as a key nosocomial pathogen in immune compromised and debilitated patients, as well as in cystic fibrosis patients. *P. aeruginosa* has always been considered to be a difficult target for antimicrobial chemotherapy. It shows a significant capacity to resist antibiotics, either intrinsically (because of constitutive expression of β-lactamases and efflux pumps, combined with low permeability of the outer-membrane) or following ahead of resistance genes (e.g., genes for β-lactamases, or enzymes inactivating aminoglycosides or modifying their target), over-
expression of efflux pumps, decreased expression of purins, or mutations in quinolone targets. Worryingly, these mechanisms are often present concurrently, thereby conferring multiresistant phenotypes.

**Streptomyces marienensis**

The streptomycetes are non-motile, non-spore forming facultative anaerobes that grow by aerobically or by fermentation. It is a gram positive microorganism and leading cause of hospital-acquired infections.

**Aspergillus niger**

*Aspergillus niger* is a fungus and one of the familiar species of the genus Aspergillus. It causes a disease called black mold on certain fruits and vegetables such as grapes, onions and peanuts and is a common contaminant of food. *Aspergillus niger* is commonly found as a saprophyte growing on dead leaves, stored grain, compost piles and other decaying vegetation. The spores are well-known and are often linked with organic materials and soil. *Aspergillus niger* is a member of the genus *Aspergillus* which includes a set of fungi that are generally considered asexual, although perfect forms (forms that reproduce sexually) have been found. *Aspergillus niger* has been found to be a opportunistic reason for infections of humans. If inhaled, in enough quantity it can cause harsh lung problems i.e., aspergillosis in humans.
Beside animal and plant pathogen, *Aspergillus niger* is also reported to produce ochratoxin A and fumonisin B2 and aflatoxins in stored commodities, which seems to be very predictable.

**Rizopus stolonifer**

*Rizopus stolonifer* is distributed threadlike Mucoralean mold. It grows rapidly at temperature between 15 to 30 °C. It has mixed distribution. It is common on bread. Thus it is also named as black bread mold. It is a cause of opportunistic infections of humans. It has great host range. Many fruits and vegetables are liable to this pathogen.

**Saccharomyces cervisiae**

*Saccharomyces cervisiae* is a yeast species. It is a microorganism for fermentation process. Thus it is passionately used in beakery, winery industry. Yeast obtained from *Saccharomyces cervisiae* also named as killer yeast.

### 2.4 Antioxidants

Antioxidants are broadly defined in the bio-medical literature as “any substance that when present at low concentration compared to those of an oxidisable substrate significantly delays or inhibits the oxidation of the substrate”. These are the substances of synthetic or natural origin
which protects the bio membranes against the Reactive oxygen species (ROS) mediated tissue damage. The possible mechanisms by which antioxidants may protect against ROS toxicity are 55-56

- Prevention of ROS formation.
- Interception of ROS attack by scavenging the reactive metabolites by converting them into less reactive metabolites and/or by enhancing resistivity of sensitive biological targets to ROS attack.
- Facilitating the repair of damage caused by ROS.
- Providing (e.g. as a co-factor or by acting to maintain in suitable Redox status) a favorable environment for the effective functioning of other anti-oxidants).

The capacity to detoxify ROS is of key importance. In human body, there is a complex combination of enzymatic and non-enzymatic function to cut the stress induced by ROS. These antioxidants may be classified as endogenous which are physiological in origin and exogenous which cannot be produced by the human body but may keep against pro-oxidant forces when administered as supplements 57,58.
2.4.1. Endogenous Antioxidants:

Even if a large number of enzymatic and non-enzymatic physiological substances are responsible to have anti-oxidant like function, the principal contributors are the enzymatic defence.

Super-Oxide Dismutase (SOD):

These are the enzymes concerned in the cellular defence against unrestrained oxidative process that catalyses the dismutation of superoxide anion radical into hydrogen peroxide (H$_2$O$_2$) and oxygen (O$_2$) and hence shrink toxic effects due to this radical or to other free radicals created from secondary reactions. In mammalian tissue two types of SOD have been described;

1. Cytosolic-Cupro-Zinc (Cu-Zn-SOD)
2. Mitochondrial – Mangano- SOD (Mn-SOD)

Glutathione:

Glutathione is central in protecting the cell against a damage of exogenous toxins. A key role is the detoxification of these elements, acting either as a nucleophile, forming conjugation with electrophilic compounds, or as a reductant in the metabolism of hydroperoxides, free radicals, and other oxidizing species. This reaction results in the oxidation of GSH to its disulphide (GSSG), which can severely
compromise host defence. Oxidation of GSH can arise in mechanism of detoxification of peroxide by glutathione peroxide. The, GSSG pattern may serve as an indicator of free radical induced oxidative stress.

Figure 3: Effect of Free Radicals in Cell Injury and Cellular Defence

**Mechanism**

**Catalase:**

A long acknowledged enzyme catalase is one of the most efficient protein catalysts known. It promotes the redox reaction. In detoxification device it detoxify hydrogen peroxide ($H_2O_2$), which is generated by SOD, it
converts hydrogen peroxide to water (H₂O) and free oxygen (O₂). Thus, catalase does not have major role in detoxification mechanism. Still its role cannot be neglected.

**Glutathione Peroxidase:**

It is chiefly responsible for the fall of GSH to its disulphide (GSSG) and there by maintaining the level of GSH in the tissue. GSSG generated in oxidation of GSH is revert back to GSH by NADPH-dependent GSSG – reductase. Additionally, it plays role in detoxification of lipid peroxy radical (LOO·), hydrogen peroxides and free radicals (R).

Thus, these antioxidant are able at pretty low concentration, to compete with other oxidizable substrate and, thus to considerably hindrance or inhibit the oxidation of these substrate. In addition to above enzymes some non-enzymatic substance such as α- tocopherol (Vitamin E), β- carotene, ascorbic acid (Vitamin C) also plays important role in evaluation of prooxidant and antioxidant mechanism.

**2.4.2. Exogenous Antioxidants:**

Many of the secondary cell byproduct of higher plants has been established by in-vitro experiments to shield against oxidative damage by inhibiting or quenching free radicals and reactive oxygen species. They are as follows;
a). Phenolic Defence compounds:
   i) Flavanoids
   ii) Phenolic compounds

b). Nitrogenous compounds:
   i) Alkaloids
   ii) Amino acids and amines

c). Carotenoids: β-carotene serves effective antioxidant and protective function against oxidative damage.

   These compounds have relatively low antioxidant activity, but when present at high concentration, can show significant reactive oxygen species (ROS) scavenging activity. Free amino acids, peptides and protein can provide as goal for oxidative attack by ROS.61

2.5 Inflammation62-68

   Inflammation is the complicated biological answer of vascular tissues to hurtful stimuli, such as pathogens, damaged cells or irritants. Inflammation is a shielding effort by host to remove the injurious stimuli also to initiate the healing for the tissue. Inflammation is not equal to infection. Even in cases where inflammation is due to infection, the two
are not synonymous. Infection is by an exogenous pathogen, while inflammation is response to the pathogen.

In the absence of inflammation, wounds and would never heal and destruction of the tissue would compromise the survival of the organism. However, chronic inflammation can also basis to several diseases\textsuperscript{62}, such as hay fever, atherosclerosis etc.

\textbf{Cause:}

- Burns
- Chemical irritants
- Frostbite
- Toxins
- Infection by pathogens
- Physical injury, blunt or penetrating
- Ionizing radiation
- Foreign bodies, including splinters and dirt
- Immune reactions due to hypersensitivity

\textbf{Clinical signal of Inflammation:}

Acute inflammation is a short-term reply, usually seen within a few minutes or hours and ceases upon the subtraction of the injurious stimulus. It is identified by five cardinal signs:
Rubor (redness),
Calor (increased heat),
Tumor (swelling),
Dolor (pain), and
Functio laesa (loss of function).

2.5.1 Types of Inflammation

Depending upon the resistance capacity of the host and duration of response, mainly three types of inflammation recognized or inflammatory response occurs in two distinct phases, each apparently mediated by different mechanisms. Based on the track and period, the inflammation can be called as:-

1) Acute: Local vasodilation and increased capillary permeability are the characteristics of acute inflammation. It is immediate and first response to injurious agents. It has three major components.

   i) Increase in blood flow

   ii) Structural changes that leads to plasma protein and leukocytes into circulation.

   iii) Accumulation of leukocyte in focus of injury.

2) Sub-Acute: This inflammation is for 1 to 6 weeks or more. The type which is neither acute nor chronic is named as sub-acute inflammation.
It lasts longer than acute inflammation. Microscopically vascular, exudative, proliferative changes of acute and chronic inflammation are present. Exudates mainly consist of eosinophils, lymphocytes, plasma cells, histocytes and fibroblasts.

3) Chronic: Chronic inflammation is considered to be prolonged duration (weeks or months) in which inflammation, tissue destruction and healing attempts are proceeding simultaneously. Chronic inflammation often begins as a low grade, smoldering after asymptomatic response and examples include rheumatoid arthritis, atherosclerosis, tuberculosis etc.

Chronic inflammation is known by;

1) Infiltration with mononuclear cells.

2) Tissue destruction.

3) Repair by connective tissue replacements.

Process of Inflammation:

The process of acute inflammation is going ahead by cells already present in tissues, mainly macrophages, histiocytes, dendritic cells, mastocytes and Kupffer cells. At the influx of an infection, burn or other injuries, these cells trigger and set free inflammatory mediators liable for the clinical signs of inflammation. Vasodilation and resulting amplified blood flow cause the redness and increased heat. Amplified permeability
of the blood vessels grades in a leakage of plasma proteins and fluid into the tissue, which manifests as swelling. Some of the free mediators such as bradykinin lift up the sensitivity to pain. The mediator molecules also alter the blood vessels to let the migration of leukocytes, principally neutrophils, outside of the blood vessels into the tissue. The neutrophils migrate along a chemotactic gradient formed by the local cells to reach the site of injury\textsuperscript{64}. The loss of function is possibly the result of a neurological reflex in response to pain.

In addition to cell-derived mediators, numerous acellular biochemical systems containing preformed plasma proteins work in equivalent to stimulate and spread the inflammatory response\textsuperscript{65}. These take account of the complement system activated by bacteria, and the coagulation and fibrinolysis systems stimulated by necrosis, e.g. a burn or a trauma.

Inflammatory mediators have tiny lives and are soon degraded in the tissue. Hence, inflammation reduces as soon as the stimulus has been removed.

The changes in acute inflammation are as follows: -

A. Exudative component

B. Cellular component
A. Exudative Component

The exudative component contains the movement of plasma fluid, containing important proteins like fibrin and antibodies, into inflamed tissue. This shift is achieved via the chemically triggered dilation and increased permeability of blood vessels, which cause a loss of blood plasma. The amplified gathering of fluid into the tissue results in it swelling.

Vascular changes: Acute inflammation is recognized by marked vascular changes, including vasodilation, increased permeability, and the slowing of blood flow, which are resultant to the actions of a range of inflammatory mediators. Vasodilation occurs at the arteriole level, continuing to the capillary level, and results in an increase in the amount of blood present, making the redness and heat of inflammation. Amplified permeability of the vessels makes passage of plasma into the tissues, with stasis due to the raise in the concentration of the cells within blood - a condition known by enlarged vessels packed with cells. Stasis allows leukocytes to travel along the endothelium, a growth critical to their recruitment into the tissues. Normal flow of the blood resists this, as the shearing force along the periphery of the vessels shifts cells in the blood into the middle of the vessel.\textsuperscript{66}
Plasma Cascade Systems:

- The activated complement system makes the increased removal of pathogens via opsonisation and phagocytosis.
- The kinin system activate proteins able of sustaining vasodilation and other physical inflammatory effects.
- The coagulation system makes a protective protein within the sites of injury.
- The fibrinolysis system, which act in conflict to the coagulation system, to balance clotting and produce several other inflammatory mediators.

Table 3: Plasma Derived Mediators

<table>
<thead>
<tr>
<th>Name(Produced by)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bradykinin (Kinin system)</td>
<td>A vasoactive protein capable to make vasodilation, increase vascular permeability, smooth muscle contraction, and pain</td>
</tr>
<tr>
<td>C3 (Complement system)</td>
<td>Cleaves to c3a and 3b. C3a initiate histamine discharge by mast cells, resulting vasodilation. C3b is able to unite to bacterial cell walls and act as an opsonin, which marks the attacker as a ambition for phagocytosis.</td>
</tr>
<tr>
<td>C5a (Complement system)</td>
<td>Causes histamine discharge by mast cells, by this means occuring vasodilation. It acts as a</td>
</tr>
<tr>
<td>Factor xll /Hageman factor (Liver)</td>
<td>A protein which circulates inactively. When stimulated by collagen, platelets, or exposed basement membranes by conformational change. When active, able to elicit three plasma systems: the kinin system, fibrinolysis system, and coagulation system</td>
</tr>
<tr>
<td>Membrane attack complex (Complement system)</td>
<td>A complex of the complement proteins C5b, C6, C7, C8, and multiple units of C9. The blend and activation of these complement proteins makes the membrane attack complex, which is liable to insert into bacterial cell walls and causes cell lysis, resulting death.</td>
</tr>
<tr>
<td>Plasmin(Fibrinolysis system)</td>
<td>Break down fibrin clots, cleave protein c3, and activate factor xii</td>
</tr>
<tr>
<td>Thrombin (Coagulation system)</td>
<td>Break the soluble plasma protein fibrinogen to produce insoluble fibrin (blood clot). Thrombin can also join to cells using the PAL1 receptor to trigger several other inflammatory responses, such as assembly of chemokines and nitric oxide.</td>
</tr>
</tbody>
</table>

**B. Cellular Component**

The cellular component contains leukocytes, which normally live in blood and must migrate into the inflamed tissue passing through extravasation for inflammation. Some act as phagocytes. Others set free enzymatic granules which hurt pathogenic invaders. Leukocytes also
release inflammatory mediators. Commonly speaking, acute inflammation is mediated by granulocytes, while chronic inflammation is mediated by mononuclear cells like monocytes and lymphocytes\(^65\).

**Leukocyte Extravasation:**

Various leukocytes are essentially concerned in the creation and continuation of inflammation. These cells must be able to attain to the site of injury from their normal position in the blood, therefore mechanisms exist to employ and express leukocytes to the right place. The mode of leukocyte association from the blood to the tissues through the blood vessels is extravasation, and can be alienated up into subsequent steps\(^66\):

**Leukocyte localisation and employment to the endothelium local to the spot of inflammation – involving margination and adhesion to the endothelial cells:**

Employment of leukocytes is receptor-mediated. The matter of inflammation, like histamine, sponsors the immediate expression of P-selectin on endothelial cell surfaces. These receptors join softly to carbohydrate ligands on leukocyte surfaces and ask them to "roll" next to the endothelial surface as bonds are complete and broken\(^66-68\).

Cytokines from hurt cells bring the emergence of E-selection on endothelial cells, which works similarly to P-selection. Cytokines also
source the appearance of integrin ligands on endothelial cells, which then after slow leukocytes down. These inadequately bound leukocytes are free to separate if not triggered by chemokines formed in injured tissue. Activation increases the affinity of bound integrin receptors for ligands on the endothelial cell surface, firmly binding the leukocytes to the endothelium\textsuperscript{66-68}.

**Movement across the endothelium (transmigration) by diapedesis:**

Chemokine gradients motivate the adhered leukocytes to transfer between endothelial cells and surpass the basement membrane into the tissues.

**Movement of leukocytes inside the tissue by chemo-taxis:**

Leukocytes cause the tissue interstitium bond to extracellular matrix proteins by expressed integrins and CD44 to shun their loss from the site. Chemoattractants becomes centre for the leukocytes to shift along a chemotactic gradient in the direction of the source of inflammation\textsuperscript{67}. 


Figure 4: Neutrophils travel from Blood Vessels to the Inflamed Tissue by Chemotaxis, where they take out Pathogens through Phagocytosis and Degranulation.

Table 4: Cell Derived Mediator

<table>
<thead>
<tr>
<th>Name</th>
<th>Type (Source)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lysosome granuls</td>
<td>Enzymes</td>
<td>These cells include a variety of enzymes which perform upon a number of functions. Granules can be classified as specific or azurophilic depending upon the contents, and are able to divide a number of substances, some of which may perhaps plasma-derived proteins which allow these enzymes to work as inflammatory mediators.</td>
</tr>
<tr>
<td>Histamine</td>
<td>Vasoactive amine (Mast)</td>
<td>Stored in preformed granules, histamine is liberated in answer to a</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>-------------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td>cells, basophils, platelets)</td>
<td>number of stimuli. It makes arteriole dilation and increased venous permeability</td>
</tr>
<tr>
<td>IFN-y</td>
<td>Cytokine (T-cells, nk cells)</td>
<td>Antiviral, immunoregulatory and anti-tumour properties. This interferon was formerly called macrophage-activating factor, and is mainly vital in the preservation of chronic inflammation</td>
</tr>
<tr>
<td>IL-8</td>
<td>Chemokine (Primarily macrophages)</td>
<td>Activation and chemoattraction of neutrophils, during a weak effect on monocytes and eosinophils.</td>
</tr>
<tr>
<td>Leukotriene B4</td>
<td>Eicosanoid (Leukocytes)</td>
<td>Able to referee leukocyte adhesion and activation, allowing them to knot to the endothelium and migrate across it. In neutrophils, it is also a powerful chemoattractant, and is able to fetch the formation of reactive oxygen species and the discharge of lysosome enzymes by these cells.</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>Soluble gas (Macrophages, endothelial cells, some neurons)</td>
<td>Potent vasodilator, relaxes smooth muscle, lowers platelet aggregation, aids in leukocyte employment, direct antimicrobial activity in high concentrations.</td>
</tr>
<tr>
<td>Prostaglandins</td>
<td>Eicosanoid (Mast cells)</td>
<td>A group of lipids which can basis vasodilation, fever and pain.</td>
</tr>
<tr>
<td>Tnf-R and il-1</td>
<td>Cytokines</td>
<td>Both affect an sufficient variety of</td>
</tr>
<tr>
<td>(Primarily macrophages)</td>
<td>cells to tempt many inflammatory reactions: fever, endothelial gene regulation, leukocyte adherence, chemotaxis, production of cytokines, and activation of fibroblasts. Prone for the systemic effects of inflammation, such as failure of appetite and amplified heart rate</td>
<td></td>
</tr>
</tbody>
</table>

**Morphologic Patterns:**

Specific patterns of acute and chronic inflammation are observed through particular situations that occur in the body, such as when inflammation took place on an epithelial surface, or pyrogenic bacteria are involved\(^{65-67}\).

- **Granulomatous Inflammation:** Known by the development of granulomas, they are the outcome of a partial but diverse numbers of diseases, which include tuberculosis, leprosy and syphilis\(^{65-66}\).

- **Fibrinous Inflammation:** Inflammation consequential in a huge raise in vascular permeability makes fibrin to move through the blood vessels\(^{65}\). If a right procoagulative stimulus is nearby, such as cancer cells, a fibrinous exudate is submited. This is normally seen in serous cavities, where the translation of fibrinous exudate
into a scar can take place among serous membranes, restraining their function\textsuperscript{66}.

- **Purulent Inflammation:** Inflammation ensuing in large quantity of pus, which has neutrophils, dead cells, and fluid. Infection by pyogenic bacteria like staphylococci is feature of this kind of inflammation\textsuperscript{67}. Large, localised summations of pus enclosed by surrounding tissues are called abscesses.

- **Serous Inflammation:** Known by the copious effusion of non-viscous serous fluid, normally formed by mesothelial cells of serous membranes, but may be derivative from blood plasma. Skin blisters express this pattern of inflammation\textsuperscript{66-67}.

- **Ulcerative Inflammation:** Inflammation near an epithelium can consequence in the necrotic loss of tissue from the surface, exposing lower layers. The following excavation in the epithelium is known as an ulcer\textsuperscript{67}.

**Inflammation Disorders:**

Abnormalities associated with inflammation comprise a large, formally distinct group of disorders which inspire a range of human diseases. The immune system is concerned with inflammatory disorders, established in both allergic reactions and numerous myopathies, with various immune system disorders resulting in abnormal inflammation.
Non-immune diseases with etiological origins in inflammatory processes are consideration to comprise cancer, atherosclerosis, and ischaemic heart disease\(^6\).

A large range of proteins are concerned in inflammation, and any one of them is open to a genetic mutation which impairs or otherwise dysregulates the normal function and expression of that protein.

Examples of disorders associated with inflammation include:

- Acne vulgaris
- Glomerulonephritis
- Autoimmune diseases
- Reperfusion injury
- Chronic inflammation
- Asthma
- Chronic prostatitis
- Hypersensitivities
- Inflammatory bowel diseases
- Pelvic inflammatory disease
- Rheumatoid arthritis


2.5.2 *In-vivo* Models of Anti-Inflammatory Activity\textsuperscript{67-68}:

The inflammatory route involves a sequence of events that can be elicited by many stimuli like infectious agents, ischemia, thermal or mechanical injury and antigen-antibody interactions. The response is associated the clinical signs of edema, erythema, hyperalgesia and pain. Inflammatory responses happen in three distinct phases, each apparently mediated by different mechanisms:

- An acute, transient phase categorized by local vasodilatation and increased capillary permeability.

- A sub-acute phase, characterized by inflammation of leukocytes and phagocytic cells.

- Chronic proliferative phase, in which tissue degeneration and fibrosis occur.

According to these phases, pharmacological methods have been developed. Methods for testing acute and sub-acute inflammations are:

a. UV erythema in guinea pigs.

b. Vascular permeability.

c. Oxazolone-induced ear edema in mice.

d. Paw edema in rats.

e. Pleurisy tests.
f. Granuloma pouch technique.

The proliferative phase is measured by methods for testing granuloma formation such as:

a. Cotton wool granuloma.

b. Glass rod granuloma.

c. PVC sponge granuloma

Models of Acute Inflammation:

- **Paw Edema**: Numerous methods are used for screening of anti-inflammatory drugs; one of the universally employed techniques is the ability of such agents to hinder the edema formed in the hind paw of the rat following injection of a phlogistic agent. Various irritants have been used, such as dextran, formaldehyde, egg albumin, brewer’s yeast, kaolin and aerosil, sulfated polysaccharides like carrageenan or naphthoylheparamine. The hind limb can be dissected at the talocrural joint and weighed. The amount of the injected paw is calculated before and after application of the irritant and the paw volume of the treated animals is compared to the controls. The value of the measurement is less dependent apparatus but much more on the irritant being chosen. Some irritants induce small lasting inflammation whereas other irritants cause the paw edema to continue over more than 24 hr$^{67}$. 
**Procedure:** Male or female Wistar albino rats with a body weight among 150 and 180 g are used. The animals are starved overnight. To assure uniform hydration, the rats accept 5 ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. 30 minutes later, the rats are challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured plethysmographically immediately after injection, again 3 and 6 h, and eventually 24 h after challenge\(^6^7\).

**Evaluation:** The increase of paw volume after 3 or 6 h is calculated as % and compared with the volume measured instantly after injection of the irritant for each animal. Treated animals show much less edema. The variation of average values between treated and control groups is calculated for every time interval and statistically evaluated. The difference at the assorted time intervals gives some hints for the duration of the anti-inflammatory effect. A dose- response curve is drawn for active drugs and ED50 values can be determined\(^6^7\).

* Pleurisy test: Pleurisy is a renowned phenomenon of exudative inflammation. In experimental animals pleurisy can be induced by several irritants, such as histamine, bradykinin, microbes, mast cell
degranulators, dextran, enzymes, antigens, prostaglandins, and nonspecific irritants, like turpentine and carrageenan\textsuperscript{68}.

**Procedure:** Male Wistar albino rats weighing 220–260 g are used. The animal is lightly anaesthetized with ether, placed on its back and the hair from skin over the ribs of the right side is isolated using animal clippers. The section is swabbed with alcohol. A small incision is made into the skin under the right arm between the seventh and eighth rib. The wound is opened and a further thin incision is made into the exposed intercostals muscle. 0.1 ml of 2\% carrageenan solution is injected to the pleural cavity via this incision. The injection needs to be made swiftly to shun the possibility of injuring the lung. The wound is closed with a Michel clip. One hour before carrageenan injection and 24 and 48 h thereafter, groups of 10 rats are treated with the standard or the test compound subcutaneously or orally. A control group receives only the vehicle of medication. The animals are sacrificed 72 hr after carrageenan injection by ether inhalation. The animal is pinned on a dissection board with the forelimbs fully extended. An incision in the skin over the xiphosternal cartilage is made to open the cartilage from overlying connective tissue. The cartilage is lifted with a forceps and a small cut is made with scissors in the body wall below to gain access into the pleural cavity. 1.0 ml of heparinized Hank’s solution is injected into the pleural cavity through this cut. The cavity is softly massaged to mix its contents. The fluid is aspirated out of the cavity using a pipette. This is made
easier if the dissection board is raised to an angle of 45–60°; the contents then pool in the corners of the cavity. The aspirated exudate is collected in a graduated plastic tube.

**Evaluation:** 1.0 ml (Hank’s solution) is subtracted from the measured volume. The values of each experimental group are averaged and compared with the control group. ED₅₀ values can be calculated using various doses. Several other parameters can be used.

- Determination of PgE2.
- Measuring the WBC number in the exudate,
- Determination of lysosomal enzyme activities,
- Determination of fibronectin,

**Erythema:** This test was planned to measure anti-inflammatory activity against erythema caused by radiation. A small spot of the back of depilated, albino guinea pig is exposed to U.V. radiation from a lamp for 20 seconds. The animal is prearranged saline or a test substance orally 30 minutes before radiation and the degree of erythema (0-4) is estimated 120 minutes later. Animals are given saline to produce erythema of degree ¾. The successful dose of erythema inhibition is defined as that of a test compound which in group of animals reduces the standard erythema to a mean response of two.
Inhibition of Ascites: Rats weighing about 300 grams are treated with a test substance and are prearranged an intraperitoneal injection of 1ml of 1.5% formalin solution 4-8 hours later the rats are sacrificed. The ascitic fluid is measured instantly. Its diminution in weight in comparison with the control group is determined.

Models of Subacute Inflammation:

Granuloma pouch technique: An aseptic inflammation resulting in huge volumes of hemorrhage exudate is elicited which resembles the subacute type of inflammation. Instead of croton oil carrageenan can be used as irritant.

Procedure: Male or female Wistar albino rats with a body weight between 150 and 200 g are used. Ten animals are taken for controls and for test groups. The back of the animals is shaved and sterilized. With a very thin needle a pneumoderma is prepared in the middle of the dorsal skin by injection of 20 ml of air under ether anesthesia. Into the resulting oval air pouch 0.5 ml of a 1% solution of Croton oil in sesame oil is injected avoiding any leak of air. 48 hr later the air is withdrawn from the pouch and 72 hr later any resulting adhesions are broken. Instead of croton oil 1 ml of a 20 % suspension of carrageenan in sesame oil can be used as irritant. Starting with the formation of the pouch, the animals are treated every day either orally or subcutaneously with the test compound or the standard. For testing local activity, the test
compound is injected directly into the air pouch at the same time as the irritant. On the 4th or the 5th day the animals are sacrificed under anesthesia. The pouch is opened and the exudate is collected in glass cylinders. Controls have an exudate volume between 6 and 12 ml, which is reduced dose dependent in the treated animals.

**Evaluation:** The average value of the exudate of the controls and the test groups is calculated. Comparison is made by statistical means. A clear dose response curve could be found.

Indomethacin was found to be effective.

- **Formalin-induced Inflammation in Rat Paw:** 0.1 ml of 2% formaldehyde is injected subcutaneously below plantar aponeurosis of right hind paw of rat. Paw volume is observed plethysmographically 15, 21 and 43 hours after injection of formalin. NSAID is given half an hour before formalin.

- **Nystatin-induced Inflammation in Rat Paw:** 0.1 ml of 6% suspension of Nystatin is injected subcutaneously under plantar neurosis in right hind paw. Paw volume is recorded plethysmographically 2, 4, 6, 24, 48, 72 hrs after nystatin. Nystatin induces inflammation reaction by labilising the lysosomal membrane.

**Models of Chronic Inflammation:**
Cotton wool granuloma: The amount of newly formed connective tissue can be measured by weighing the dried pellets after removal. More intensive granuloma formation has been seen if the cotton pellets have been impregnated with carrageenan.

Procedure: Male Wistar albino rats with an average weight of 200 g are anaesthetized with ether. The back skin is shaved and disinfected with 70% ethanol. A cut is prepared in the lumbar region. A blunted forceps forms subcutaneous tunnels and a sterilized cotton pellet is placed on both sides in the scapular region. The pellets are either standardized for use in dentistry weighing 20 mg or pellets formed from raw cotton, which produce a more pronounced inflammation than bleached cotton. The animals are treated for 7 days subcutaneously or orally. Then, the animals are sacrificed, the pellets prepared and dried until the weight remains constant. The net dry weight, i.e. after subtracting the weight of the cotton pellet is determined.

Evaluation: The average weight of the pellets of the control group as well as of the test group is calculated. The % change of granuloma weight relative to vehicle control group is determined.

Glass rod granuloma: Of the newly formed connective tissue not only wet and dry weight, but also chemical composition and mechanical properties can be measured.
Procedure: Glass rods with a diameter of 6 mm are cut to a length of 40 mm and the ends smoothed by flame melting. They are sterilized before implantation by boiling in water. Male Wistar albino rats with an initial weight of 130 g are anaesthetized with ether, the back skin shaved and disinfected. From an incision in the caudal region a subcutaneous tunnel is formed in cranial direction with a closed blunted forceps. One glass rod is introduced into this tunnel finally lying on the back of the animal. The incision wound is closed by sutures. The animals are isolated in separate cages. The rods insitu remain for 20 or 40 days. Treatment with drugs is either during the whole period or during the last 10 or 20 days. At the end the animals are sacrificed under CO₂ anaesthesia. The glass rods are prepared together with the surrounding connective tissue, which forms a tube around the glass rod. By cut at one end the glass rod is extracted and the granuloma sac inverted forming a plain piece of pure connective tissue. Wet weight of the granuloma tissue is recorded. The specimens are kept in a humid cavity until further analysis. For measurement of the mechanical properties the specimens are fixed into the clamps of the Instron (R) instrument allowing a gauge length of 30 mm. The load until break is recorded with a crosshead speed of 50 mm/min. In order to calculate tensile strength (N/mm²), the value of load at rupture (N) is divided by cross sectional area (measured as volume = wet weight divided by length). Finally, the granuloma tissue is dried and the dry weight is recorded. In addition, biochemical analyses,
such as determination of collagen and glycosaminoglycans, can be performed.

**Table 5: Medicinal Plants Used for the Treatment of Analgesic and Anti-Inflammatory Activity**

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Family</th>
<th>Parts Used</th>
<th>Chemical Constituent</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Manilkara zapota</em> (Chickoo)</td>
<td>Sapotaceae</td>
<td>Leaves</td>
<td>Alkaloids, flavonoids, steroids, phenolic Compounds</td>
<td>Analgesic</td>
</tr>
<tr>
<td><em>Scoparia dulcis</em> L (Mithi patti)</td>
<td>Scrophulariaceae</td>
<td>whole herb</td>
<td>Alkaloids, carbohydrates, glycosides &amp; tannins</td>
<td>Analgesic</td>
</tr>
<tr>
<td><em>Ficus racemosa</em> (Udumbar)</td>
<td>Moraceae</td>
<td>Fruits</td>
<td>tannins, gums, flavonoids &amp; alkaloids</td>
<td>Analgesic</td>
</tr>
<tr>
<td><em>Allium stracheyi</em> (Pharna)</td>
<td>Liliaeceae</td>
<td>Leaves</td>
<td>steroids, alkaloids, saponin</td>
<td>Analgesics</td>
</tr>
<tr>
<td><em>Mitragyna parvifolia</em> (kadam)</td>
<td>Rubiaceae</td>
<td>Fruits</td>
<td>pyroliquene acid, methyl acetate, ketones and aldehydes</td>
<td>Anti-inflammatory, Analgesics</td>
</tr>
<tr>
<td><em>Butea monosperma</em> (Palash)</td>
<td>Fabaceae</td>
<td>Leaves</td>
<td>Flavonoids, chalcones, tannins.</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td><em>Tectona grandis</em> (sagwan)</td>
<td>Vervenaceae</td>
<td>Leaves</td>
<td>quinones, steroids, glycosides, flavonoids, alkaloids, saponin</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td><em>Nyctanthes arbor-tristis</em></td>
<td>Oleaceae</td>
<td>Bark</td>
<td>flavonol glycosides, β -sitosterol, nycanthnic</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td>(Shefali)</td>
<td>Family</td>
<td>Part</td>
<td>Constituents</td>
<td>Property</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------------</td>
<td>------------</td>
<td>------------------------------------------------------------------------------</td>
<td>------------------------</td>
</tr>
<tr>
<td><em>Acacia catechu</em> (Katha)</td>
<td>Leguminosae</td>
<td>bark and stem</td>
<td>Tannins catechin, quercetin, catechuic acid.</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td><em>Clerodendrum phlomidis</em> (Arni)</td>
<td>Verbanaceae</td>
<td>Stem bark</td>
<td>Alkaloids, glycosides, saponins, tannins.</td>
<td>Analgesic</td>
</tr>
<tr>
<td><em>Phyllanthus niruri</em> (Gulf-leaf flower)</td>
<td>Phyllanthaceae</td>
<td>whole plant</td>
<td>Flavonoids, sterols, alkaloids, phyllanthin, hypophyllanthin</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td><em>Sterculia foetida</em> (Jangli badam)</td>
<td>Sterculiaceae</td>
<td>Seeds</td>
<td>Fat, cycloprenoid fatty acids.</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td><em>Amaranthus spinosus</em> (Prickly amaranth)</td>
<td>Amaranthaceae</td>
<td>whole plant</td>
<td>α-spinasterols octacosanoate and saponin.</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td><em>Hibiscus tiliaceus</em> (Beach Hibiscus)</td>
<td>Malvaceae</td>
<td>Leaves</td>
<td>Vanillic acid, syringic acid, β-sitosterol, Quercitin etc.</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td><em>Calotropis giganteas</em> (Crown flower)</td>
<td>Asclepiadaeceae</td>
<td>Leaves</td>
<td>Calotropnaphthalene, terpenes.</td>
<td>Anti-inflammatory</td>
</tr>
<tr>
<td><em>Holarrhena antidysenterica</em> (Indrajao)</td>
<td>Apoynaceae</td>
<td>Bark</td>
<td>Alkaloid, Tannins &amp; Flavanoids</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td><em>Tridex procumbens</em> (Ghamra)</td>
<td>Asteraceae</td>
<td>Leaves</td>
<td>flavonoids, procumbentin and quercetin, β-sitosterol</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td><em>Cissus rependa</em> (Pani bel)</td>
<td>Vitaceae</td>
<td>Root, Stem</td>
<td>Alkaloids, glycosides, saponins, tannins.</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Part Used</td>
<td>Active Components</td>
<td>Medical Properties</td>
</tr>
<tr>
<td>------------------------------------------------</td>
<td>----------</td>
<td>----------------</td>
<td>----------------------------------------------------------------------------------</td>
<td>--------------------------</td>
</tr>
<tr>
<td><em>Kaempferia galangal</em> (Aromatic ginger)</td>
<td>Zingiberaceae</td>
<td>fresh rhizome</td>
<td>ethyl-p-methoxycinnamate, methylcinnamate, Carvone etc</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td><em>Tanacetum artemisioides</em> (Paloyo Zoon)</td>
<td>Asteraceae</td>
<td>whole plant</td>
<td>Flavonoids</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td><em>Chococca brachiata</em></td>
<td>Rubiaceae</td>
<td>Root</td>
<td>Steroids, phenolic compounds, ligans</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td><em>Cynara scolymus</em> (Globe artichoke)</td>
<td>Asteraceae</td>
<td>Leaves</td>
<td>Sesquiterpenes, flavone glycosides, volatile oil.</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td><em>Marsypianthes chanaedrys</em> (Konmonmi mawon)</td>
<td>Lamiaceae</td>
<td>Leaves</td>
<td>Essential oil, germacrene D, betacaryophyllene</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td><em>Dorstonia brasiliensis</em> (Carapia)</td>
<td>Moraceae</td>
<td>Root</td>
<td>Monoterpenoid substituted furocoumarin, phytosterol.</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td><em>Sida acuta</em> (Bariara)</td>
<td>Malvaceae</td>
<td>whole plant</td>
<td>alkaloids, flavanoids, steroids, tannins, terpenoids</td>
<td>Analgesic</td>
</tr>
<tr>
<td><em>Stylosanthes fruitcosa</em> (Saillekampa)</td>
<td>Papilionaceae</td>
<td>whole plant</td>
<td>alkaloids, flavanoids, saponins, phytosterols, glycosides.</td>
<td>Analgesic</td>
</tr>
<tr>
<td><em>Toona celiata</em> (Tun)</td>
<td>Meliaceae</td>
<td>Heart wood</td>
<td>Phytosterols, coumarins, carbohydrates.</td>
<td>Analgesic</td>
</tr>
<tr>
<td><em>Kyllinga monocephala</em> (Nirbishi)</td>
<td>Cyperaceae</td>
<td>Leaves</td>
<td>Alkaloids, glycosides, saponins, tannins.</td>
<td>Analgesic</td>
</tr>
</tbody>
</table>
2.6 Analgesics\textsuperscript{69-73}

Analgesics are members of the group of drugs used to relieve pain. Analgesic drugs act in diverse ways on the peripheral and central nervous systems; they include paracetamol (also known as acetaminophen), the non-steroidal anti-inflammatory drugs (NSAIDs) such as the salicylates, and opioid drugs such as morphine and tramadol.

**Pain:** Pain is the unpleasant and aversive feeling common to such incidences as stubbing a toe, burning a finger, adding iodine on a cut and bumping the funny bone. The International Association for the study of pain defines pain as "an unpleasant sensory and emotional experience associated with actual or potential tissue damage, or described in terms of such damage". Pain motivates us to withdraw from injurious or potentially hurtful situations, protect the damaged body part.

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Family</th>
<th>Part Used</th>
<th>Constituents</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baliospormum Montanum</td>
<td>Euphorbiaceae</td>
<td>roots</td>
<td>beta-sitosterol, triterpenoids, flavonoids</td>
<td>Analgesic</td>
</tr>
<tr>
<td>Mangifera indica (Am)</td>
<td>Anarcardiaceae</td>
<td>Leaves</td>
<td>Flavonoids, polyphenolics, triterpenes, tannins</td>
<td>Anti-inflammatory, Analgesic</td>
</tr>
<tr>
<td>Carpolobia lutea (cattle stick)</td>
<td>Polygalaceae</td>
<td>Roots</td>
<td>tannins, saponins, flavonoids, cardiac glycosides, terpenes</td>
<td>Analgesic</td>
</tr>
</tbody>
</table>
while it heals, and evade those scenarios in the future. It is initiated by stimulation of nociceptors in the peripheral nervous system, or by damage to or defect of the peripheral or central nervous systems. Most pain cure promptly once the painful stimulus is disconnected and the body has healed, but sometimes pain continues although removal of the stimulus and noticeable healing of the body; and sometimes pain occurs in the absence of any assertive stimulus, damage or pathology. Public support, social values, hypnotic suggestion, enthusiasm in sport or war, diversion, and assessment can all significantly amend pain’s intensity and unpleasantness.

**Historical Aspect of Pain**

Descartes’ (Paris, 1644) concept of the pain pathway: "If for example fire comes close to the foot, the microscopic particles of this fire, which as you know shift with great velocity, have the command to set in motion the mark of the skin of the foot which they touch, and by this means pulling upon the delicate thread, which is fond of to the spot of the skin, they open up at the similar instant the pore, against which the delicate thread ends, just as by pulling at one end of a rope one makes to strike at the same instant a bell which hangs at the other end."

The nature of pain has been the matter of bitter argument since the turn of the century. There are at present two opposing theories of pain: (i) specificity theory, which holds that pain is a specific modality
like idea or hearing, "with its personal central and peripheral apparatus", and (ii) pattern theory, which maintains that the nerve impulse pattern for pain is created by intense stimulation of nonspecific receptors since "there are no specific fibers and no specific endings". Both theories derive from earlier concepts projected by von Frey and Goldscheider in 1894.

Specificity theory states that a mosaic of particular pain receptors in body tissue projects to a pain center in the brain. It maintains that free nerve endings are pain receptors and generate pain impulses that are carried by A-delta and C fibers in peripheral nerves and by the lateral spinothalamic tract in the spinal cord to a pain center in the thalamus. Clinical confirmation: The pathological pain states of causalgia (a cruel burning pain that may effect from a partial lesion of a peripheral nerve), phantom limb pain (which may occur after elimination of a limb), and the peripheral neuralgias (which may happen following peripheral nerve infections / degenerative diseases) provide a dramatic refutation of the concept of a fixed, direct-line nervous system.

Psychological facts suggesting that the amount and quality of seeming pain are determined by many psychological variables: 1) Beecher in 1959 has found that most American soldiers wounded at the Anzio beachhead "totally denied pain from their wide wounds or had so slight that they did not desire any medication to relieve it" apparently because they were thrilled at having escaped alive from the
2.) Pavlov’s (1927, 1928) dogs that received electric shocks, burns, or cuts, followed constantly by the presentation of food, ultimately responded to these stimuli as signals for food and failed to show "even the least and most subtle" signs of pain.

In the seek for peripheral fibers that react exclusively to high-intensity stimulation, Hunt and McIntyre (1960) found only 7 out of 421 myelinated a fibers, and Maruhashi et al (1952) found 13 out of several hundred. Douglas and Ritchie (1957) were unsuccessful to find any high-threshold C fibers, while Iggo (1958) found a little. These data suggest that a small number of specialized fibers may be present that respond only to concentrated stimulation.

The nature of the field of central cells remains vague even though the large number of single-cell studies. Central cells that respond entirely too noxious stimuli have also been reported; Poggio and Mountcastle’s (1960) study of such cells in the posterior thalamus in anesthetized monkeys. Yet Casey (1964), who has recently confirmed that posterior thalamic cells respond solely to noxious stimuli in the drowsy or sleeping monkey, found that the same cells also signaled information in response to gentle tactile stimulation when the animal was awake.

Pattern Theory: As a reaction against the psychological assumption in specificity theory, new theories have been proposed which can be grouped under the general heading of "pattern theory." Goldscheider,
initially one of the champions of von Frey’s theory, was the first to propose that stimulus intensity and central summation are the critical determinants of pain. Two kinds of theories have emerged from Goldscheider’s concept.

Livingston (1943) was possibly the first to suggest specific neural mechanisms to report for the remarkable summation phenomena in clinical pain syndromes. He projected that intense, pathological stimulation of the body sets up rich circuits in spinal internuncial pools, or evokes spinal cord activities such as those reflected by the “dorsal root reflex”.

One of the prime features of inflammatory states is that normally bland stimuli produce pain. Since the publication of the Melzack- Wall gate control theory in 1965, it has been generally appreciated that the nervous system exhibits a range of responses according to different conditions (‘neural plasticity’).

Gate Control Theory of Pain: Encouragement of the skin evokes nerve impulses that are transmitted to 3 spinal cord systems: the cells of the substantia gelatinosa in the dorsal horn, the dorsal-column fibers that project toward the brain and the first central transmission (T) cells in the dorsal horn. (i) the substantia gelatinosa functions as a gate control system that modulates the afferent patterns before they influence the T cells; (ii) the afferent patterns in the dorsal column
system act, in part at least, as a central control trigger which activates selective brain processes that affect the modulating properties of the gate control system; and (iii) the T cells activate neural mechanisms which contain the action system responsible for response and perception. This theory states that pain phenomena are determined by interactions between these three systems.

The utilization of willow barks and leaves to reduce fever has been attributed to hypothesis but was most clearly acknowledged by Edmund Stone in a 1763 letter to the president of The Royal Society. Salicin was found out in 1829 by Leroux. And Pinn found out salicylic acid in 1836, and by 1874, it was being manufactured industrially. It quickly was being adapted for rheumatic fever, gout and as a general antipyretic. However, its nasty taste and adverse GI effects made it hard to tolerate for more than small periods. Hoffmann (Bayer laboratories) in 1899 started testing acetylsalicylic acid (ASA) in animals – the 1st time a drug was experimented on animals in an industrial setting- and proceeded soon thereafter to human studies and the marketing of aspirin.

**Time interval of Pain**

Pain is usually short-lived, exist only until the noxious stimulus is removed or the underlying damage or pathology has healed, but some painful situation, such as rheumatoid arthritis, peripheral neuropathy, cancer and idiopathic pain, may carry on for years.
Pain that exist a long time is called chronic, and pain that terminate quickly is called acute. Traditionally, the dissimilarity between acute and chronic pain has depend upon an arbitrary interval of time from beginning; the two most commonly used markers being 3 months and 6 months since the begining of pain, though some theorists and researchers have positioned the transition from acute to chronic pain at 12 months. Others relate the phrase acute to pain that lasts less than 30 days, chronic to pain of more than 6 months duration, and subacute to pain that lasts from 1 to 6 months. A popular substitute definition of chronic pain as concerning no arbitrarily fixed durations is "pain that leads beyond the expected period of healing."

**System and Region**

Pain can be divided according to its location in the body, as in head, lower backache and pelvic regen or according to the body system concerned,

- **Myofascial** (emanating from skeletal muscles or the fibrous cover surrounding them)
- **Causalgic** ("burning" pain in the skin of the arms or sometimes legs; thinking to be the product of peripheral nerve damage)
- **Neurologic** (caused by damage to or malfunction of any part of the nervous system)
- Rheumatic (emanating from the joints and surrounding tissue)
- Vascular (pain from blood vessels).

**Etiology of Pain:**

The classification by etiology simply differentiates "**somatogenic**" pain from "**psychogenic**" pain. Portenoy divided somatogenic pain into "nociceptive" and "neuropathic. Nociceptive pain is subdivided into "superficial" and "deep" - and deep pain is further divided into "deep somatic" and "visceral". Superficial pain is caused by injury to the skin or superficial tissues and is usually a sharp, well-defined, clearly localized pain. Examples of injuries that produce superficial pain include minor wounds and minor (first degree) burns.

Deep somatic pain sarts in ligaments, bones, blood vessels, tendons, fasciae and muscles, and is a dull, aching, poorly-localized pain; examples include broken bones, sprains and myofascial pain. Visceral pain starts in the organs and is usually more aching or cramping than somatic pain. Visceral pain may be well-localized, but is often difficult to locate, and several visceral regions produce "referred" pain when injured, where the sensation is situated in an area completely isolated to the site of injury. Nociceptive pain may also be classified to the noxious stimulation. The most common categories are "thermal", "mechanical" and "chemical".
Neuropathic pain is divided into "peripheral" and "central". Peripheral neuropathic pain is often described as burning, electrical, stabbing, tingling or pins and needles. Bumping the "funny bone" starts peripheral neuropathic pain.

**Mechanism of Pain Perception and Nociceptive Afferent Neurons:**

Trigeminal and dorsal root ganglia (DRG) possess nociceptor cell bodies which give rise to myelinated Aδ and unmyelinated C fibers. Under normal conditions pain is coupled with electrical activity in small diameter primary afferent fibers of peripheral nerves. These nerves have sensory endings in peripheral tissues and are initiated by stimuli of various kinds (mechanical, thermal, chemical). They are differentiated from other by mechanical and thermal receptors with their higher threshold. Since they are normally stimulated only by stimuli of noxious intensity, sufficient to cause some degree of tissue damage. Stimuli sufficient to excite these small afferent fibres also initiate a painful sensation.

Non-myelinated C fibres have low conduction velocities (<1m/s). Aδ fibres conduct more speedily but respond to similar peripheral stimuli. Aδ and C fibers transduce and spread noxious stimuli to the dorsal horn of the spinal cord from where these stimuli are transmitted to the brain. At the level of the spinal cord and at supraspinal sites, various
neurotransmitters come into play, which together with environmental and cognitive factors, supply to the eventual sensation of pain.

With many pathological conditions, tissue injury is the immediate cause of the pain, and this results in the local release of a variety of chemical agents, which are assumed to act on the nerve terminal, either activating them directly or enhancing their sensitivity to other forms of stimulation.

Nociceptors are receptors that reply to painful stimuli and are thin nerve fibers in the skin, muscle, and other body tissues, that, when motivated, carry pain signals to the spinal cord and brain. Normally, nociceptors only react to strong stimuli such as a pinch. However, when tissues become injured or inflamed, as with a sunburn or infection, they release chemicals that make nociceptors much more sensitive and cause them to transmit pain signals in response to even gentle stimuli such as breeze or a caress. This condition is called allodynia, a state in which pain is produced by innocuous stimuli.

**Some Chemical Mediators for the Nociceptive Pathways:**

a. **Capsaicin:** Capsaicin (the stuff in chilli peppers that gives them their pungency), selectively excites nociceptive nerve terminals, making intense pain if injected into the skin or applied to sensitive structures
such as cornea. It gives this effect by binding to vanilloid receptor (ligand gated cation channel).

b. **Kinins:** The chief active kinins are Bradykinin and Killidin, 2 closely related peptides created under conditions of tissue injury by the proteolytic cleavage of the active kinins from a precursor protein contained in the plasma.

Bradykinin is a strong pain producing substance, acting partially by release of prostaglandins, which powerfully enhance the direct action of bradykinin on the nerve terminals. It acts by connecting with specific G-protein coupled receptors and produce various intracellular messengers. Prostaglandins do not themselves cause pain, but they strongly boost the pain producing effect of other agents such as 5-HT or bradykinin.

Prostaglandins of the E and F series are released in inflammation and also during tissue ischemia. They sensitize nerve terminals to other agents partly by inhibiting potassium channels and partly by facilitating-through second messenger mediated phosphorylation reactions- the cation channel opened by noxious agents.

c. **Peripheral mediators:**

Various substances and metabolites are released from damaged or ischemic cells or inflamed tissue including 5-HT, histamine, lactic acid,
ATP and K⁺, many of which influence nociceptive nerve terminals. 5-HT reasons excitation, but research with antagonists proposes that it plays at most a small role. Histamine causes itching than real pain. Both 5-HT and Histamine are known to be free locally inflammation.

**Transmitters and modulators in the nociceptive pathway are:**

Tachykinins like Neurokinin (NK) A and B, substance P, are endogeneous peptides which act as transmitters and modulators for nociceptive pathway and are freed at both the central and the peripheral terminals when the neurons are activated. Release of peptides of peripheral terminals of these neurons is reflection to play a part in neurogenic inflammation. Nociceptive transmission and neurogenic inflammation are mediated mainly through Tachykinin (NK) receptors.
Figure 5: Chemical Mediators in the Nociceptive Pathways.

Other central mediators are Glutamate freed from primary afferent neurons, Gamma amino butyric acid freed by spinal cord inter neurons inhibits transmitter discharge by primary afferent terminals in the dorsal horn. 5-HT, Noradrenaline, and Adenosine plays a twin role in regulating nociceptive transmission, activation of A1 receptors causing analgesia while activation of A2 receptors does the response reverse.

Supervision of Pain:

Analgesics are the class of drugs which includes most painkillers, such as aspirin, acetaminophen, and ibuprofen. Nonprescription or over-
the-counter pain relievers are commonly used for treating mild to moderate pain. Different types of drugs used to relieve pain, include:

Opioid / narcotic / morphine like analgesics.

Figure 6: Inflammatory Mediators Derived from Phospholipids with their Actions and the Sites of Action of Anti-inflammatory Drugs.

2.6.1 Animal Models to Screen Analgesic Activity

A. Central Analgesic Activity

_In-vivo_ Methods:
1. HAFFNER’S tail clip method

2. Radiant heat method

3. Hot plate method

4. Tail immersion method

5. Electrical stimulation of the tail

6. Grid shock test

7. Formalin test in rats

B. **Peripheral Analgesic Activity:**

1. Writhing test

2. Pain in inflamed tissue (RANDALL-SELITTO-test)

3. Antagonism against local effects of bradykinin.

4. Effect of analgesics on spinal neurons.

**Screening Methods of Analgesic Action:**

*In vivo methods for testing central analgesic activity:*

**HAFFNER’S tail clip method:** The method was explained as early as 1929 by Haffner who experimented the raised tail in mice treated with morphine or parallel opioid drugs and found the tail after drug ingestion
to be less sensitive to injurious stimuli. He already established the high sensitivity of this method to morphine. Since then, the method has been used and modified by many authors. The test does not need any sophisticated equipment but a skilled, preferably “blind”, observer. Peripheral analgesics of the salicylate type are not detected by this test.

**Models Using Thermal Stimulus:**

**Radiant heat method:** Formerly, the method was explained by Schumacher et al. (1940), Wolff et al. (1940) for quantitative dimensions of pain threshold in man against thermal radiation and for evaluation of analgesic activity of opiates. Later on, the method has been used by many authors to assess analgesic activity in animal experiments by measuring drug-induced changes in the sensitivity of mice or rats to heat stress applied to their tails. The test is very useful for differentiate between centrally acting morphine-like analgesics and non-opiate analgesics. The radiant heat test on the tail of mice is very useful to estimate the efficacy and potency of central acting analgesic drugs. With pyrazolones ED50 values still can be planned but these are achieved only with relatively high doses. Compounds like acetylsalicylic acid and phenyl-acetic acids prove only slight effects making it difficult to calculate ED$_{50}$ values.

**Hot plate method:** The paws of mice and rats are very susceptible to heat at temperatures which are not destructive for the skin. The
responses are removal of the paws, jumping and licking of the paws. The time till these responses come about is prolonged after ingestion of centrally acting analgesics, whereas peripheral analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses. The hot plate test has been used by many investigators and has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. Mice as well as rats have been used. The method has the problem that sedatives and muscle relaxant or psychotomimetics cause false positives, while mixed opiate agonists-antagonists showed variable results. The validity of the test has been observed even in the presence of substantial impairment of motor performance. Mixed opiate agonists-antagonists can be examined if the temperature of the hot plate is lowered to 49.5 °C.

**Tail immersion test:** The method has been observed to be selective for morphine-like compounds. It is based on the finding that morphine-like drugs are selectively able to prolong the reaction time of the typical tail-withdrawal reflex in rats induced by immersing the end of the tail in warm water of 55 °C. The test is useful to differentiate central opioid like analgesics from peripheral analgesics.

**Electrical stimulation of the tail:** As the tail of mice is found to be sensitive to any stimulus, electrical stimulation has been studied. The stimulus can be either the duration of the electric shock or an increase
in the electric current. The outcome of central analgesics can be clearly studied; also the activity of peripheral analgesics at higher doses can be found out.

**Grid shock test:** The electric grid shock test in mice has been described as an alteration of an earlier approach to compute the analgesic properties by the “Flinch-jump” process in rats. The alteration of the method showed an effect not only of morphine but also of acetylsalicylic acid which is not easily picked up by other tests based on inspiration by physical means.

**Tooth pulp stimulation:** The method has been foremost described for testing central analgesic activity in rabbits and since then functional by several authors to various animal species. Stimulation of the tooth pulp makes characteristic reactions, such as chewing, biting, licking and head flick. Central analgesics, especially opioid agonists, have been seen to be very active in this test. In addition, non-opiate analgesics like ketamine and peripheral analgesics like pyrazolone derivatives show a positive response.

**Monkey shock titration test:** Generally, painkiller tests in rats and mice result in correlation with the analgesic activity of a drug in man. To clarify the mode of action in more detail and to find a suitable dosage therapy in man, trials in monkeys may be necessary. The monkey shock titration test may be used for final assessment of a new drug before
administration to man. For screening activities the procedure cannot be optional since the test is too time intense and the apparatus too complicated. Furthermore, higher animals such as monkeys should only be used if absolutely necessary.

**Models using chemical stimulus:**

**Formalin test in rats:** The formalin test in rats has been projected as a chronic pain model which is sensitive to centrally active analgesic agents. The formalin test identifies mainly centrally active drugs, whereas peripherally acting analgesics are almost hopeless. Therefore, the formalin test may allow variation between inflammatory and non-inflammatory pain, a rough classification of analgesics according to their site and their mechanism of action.

**Peripheral Analgesic Activity**

**Writhing tests:** Pain is developed by injection of irritants (eg. acetic acid) into the peritoneal cavity of mice. The animals act in response with a characteristic stretching behavior called writhing. The test is suitable to sense analgesic activity although some psychoactive agents also prove activity. An irritating agent such as phenylquinone or acetic acid is introduced intraperitoneally to mice then the stretching reaction is examined. The reaction is not specific for the irritant. In this test both central and peripheral analgesics are detected. The test, therefore, has
been utilised by many investigators and can be known as a simple screening method. However, it has to be studied that other drugs such as clonidine and haloperidol also show a prominent activity in this test. Due of the lack of specificity, caution is necessary in interpreting the results, till other tests have been performed. Yet, a good relationship exists in the potencies of analgesics in writhing assays and their clinical potencies.

**Pain in inflamed tissue: (RANDALL-SEILITTO-test):** This method for determining analgesic activity and is based on the principle that inflammation enhances the sensitivity to pain and that this sensitivity is subject to modification by analgesics. Inflammation reduces the pain reaction threshold and this low pain reaction threshold is readily increased by non-narcotic analgesics of the salicylate-amidopyrine type as well as by the narcotic analgesics. Brewer’s yeast has been used as an initiator for inflammation which increases pain after pressure.

### 2.7 Hepatoprotectives\(^{74-88}\)

**Anatomy of Liver**
The liver is the principal gland in the human body, weighing approximately 3 pounds and takes a large section habitually on the right
side of the body, under the diaphragm and following ribs 5 to 10. The liver is alienated into 4 lobes: right, left, caudate, and quadrate. The right and left lobes are the leading, while the caudate and quadrate are smaller and located posteriorly. Two ligaments are visible anteriorly. Superiorly, the falciform ligament separates the right and left lobes. Inferior to the falciform ligament is the round ligament, which protrudes from the liver slightly. Also noticeable anteriorly on the most inferior portion of the right lobe is the gallbladder.

Posteriorly, various further interesting structures are evident. The caudate lobe is sited superiorly, roughly between the right and left lobes. Nearer to the caudate lobe is the sulcus for the inferior vena cava. Just inferior to the caudate lobe is the porta hepatis, where the hepatic artery and hepatic portal vein penetrate the liver. The portal vein carries nutrient blood from the digestive system. Inferior to the porta hepatis is the bile duct which leads back to the gallbladder. Finally, the hepatic vein, where post-processed blood leaves the liver, is found inferior and adjacent to the sulcus for the inferior vena cava. The liver is held on position by a system of mesenteries posteriorly, and is also attached to the diaphragm via the falciform ligament. Also, most of the liver is enclosed by visceral peritoneum.
The fundamental functional unit of the liver is the liver lobule. A single lobule is about the size of a sesame seed and is roughly hexagonal in shape. The primary structures in a lobule include:

- Central vein
- Plates of hepatocytes
- Portal triads at each corner of hexagon
- Liver sinusoids that run from the central vein to the portal triads
- Hepatic macrophages (Kupffer cells)
- Bile canaliculi (“little canals”) – created between walls of adjacent hepatocytes
- Space of Disse – a small space among the sinusoids and the hepatocytes

The portal triads consist of three vessels: a hepatic portal arteriole, a hepatic portal venule, and a bile duct. The blood from the arteriole and the venule both stream in the parallel direction – through the sinusoids toward the central vein, which ultimately leads to the hepatic vein and the inferior vena cava. Secreted bile flows in the opposite direction – through the bile canaliculi away from the central vein, toward the portal triad, and exiting via the bile duct. As blood flows through the sinusoids and the space of Disse toward the central vein, nutrients are
processed and stored by the hepatocytes, and worn out blood cells and bacteria are engulfed by the Kupffer cells\textsuperscript{76}.

The liver has 5 cell types: hepatocytes, Kupffer cells, sinusoidal endothelial cells, bile duct epithelial cells, and Ito cells\textsuperscript{76}.

Hepatocytes signify 60\% of the liver’s cells, and about 80\% of the liver’s total cell mass. The majority of the liver’s synthetic and metabolic capabilities stem from the work of hepatocytes\textsuperscript{75}. Hepatocytes are arranged in plates only a single cell thick. Blood flowing toward the hepatic vein within the space of Disse passes both exposed surface areas of the hepatocyte plates, and toxins and nutrients within the blood are extracted by the hepatocytes\textsuperscript{76}. Kupffer cells are macrophages that reside in the sinusoids. These cells help clear out old red blood cells and bacteria. They also break down heme (the iron-containing pigment in hemoglobin) into bilirubin, which then becomes one of the chief pigments of bile. A later by-product of bilirubin gives feces its characteristic brown color. Sinusoidal endothelial cells are fenestrated, meaning they have large pores that allow most proteins to pass freely through the sinusoidal endothelium into the space of Disse, where they can make direct contact with hepatocytes. The pores are also bi-directional, meaning that proteins created by the liver and other substances stored or processed by the liver can also be passed back into the blood. Bile duct epithelial cells line the interlobular bile ducts within the portal triads\textsuperscript{77}. Its cells are
found in the space of Disse. They are important because when the liver is injured, the Ito cells transform into cells that produce collagen, which leads to liver fibrosis. If this occurs on a large scale, it can lead to cirrhosis of the liver. Cirrhosis is a serious disease of the liver.

Figure 9: Transport Polarity of Normal Hepatocytes and Bile-duct Epithelial Cells (Cholangiocytes).

Physiology of Liver

The liver has 4 essential functions:

1) Synthesis of several proteins that circulate in the blood. These include albumin, coagulation factors, alpha1-antitrypsin, very low density lipoprotein, and many others.

78.
2) Stores nutrients for afterward use. The liver balances the supply of nutrients with demand. For example, the liver stores glucose as glycogen, and converts it back to glucose as desired. If the supply of glycogen is depleted, the liver can also synthesize glucose from amino acids, lactate, and glycerol, while this is less efficient than breaking down glycogen into glucose. Furthermore, the liver metabolizes fatty acids, cholesterol, and amino acids. When there is a surplus of glucose in the bloodstream, the liver can convert excess glucose and amino acids into fatty acids for storage. The liver both synthesizes cholesterol and removes it from circulation. Finally, the liver can synthesize non-essential amino acids when needed by the body.

3) Detoxification and elimination of toxic substances. Toxins are detoxified by the liver’s ability to metabolize lipophilic compounds. These compounds (bound to albumin) enter the liver sinusoids and then the area of Disse. Enzymes in the hepatocytes (cytochrome P-450 enzymes) are involved in the metabolism of the lipophilic compounds, which include toxins and many drugs.

4) Production of bile. Bile acts as a detergent, and cleaves fats down into smaller parts so they can be digested in the small intestine. Bile also provides a manner for the liver to remove wastes, including bilirubin, cholesterol, and toxins. Bile is produced in the biliary canaliculi, which drain into the interlobular bile ducts. These ducts then merge with other
ducts, forming larger intermediate ducts, which eventually merge into the right and left hepatic ducts, which themselves merge into the common hepatic duct, which merges with the cystic duct from the gallbladder, finally forming the common bile duct, which empties into the small intestine.

**Liver Diseases:**

One way to categorize liver disorders is by their duration. A chronic disorder stay for more than 6 months; a subacute disorder stay for 3 to 6 months; while an acute disorder takes over a period less than 3 months. A very harsh disorder that leads to liver failure within 6 weeks is termed fulminant. Some of the common disorders of the liver include cirrhosis, viral hepatitis, alcoholic liver disease, hemochromatosis, and liver cancer.

1. **Jaundice**
   
a) **Haemolytic Jaundice**

b) **Hepatocellular Jaundice**

c) **Obstructive Jaundice**

2. **Hepatitis**

Viral hepatitis is of five types namely, hepatitis A, B, C, D and E.
3. **Cirrhosis**

The inflammation and damage of parenchyma of liver is known as cirrhosis of liver.

4. **Tumours of Liver**

a) **Benign tumors**

i) Benign haemangioma  

ii) Cysts  

b) **Malignant tumors**

i) Secondary metastasis is the most common tumors. It may be from breast, lungs and colon.  

ii) Primary tumours  

5. **Hepatocellular Carcinoma**

It is the most common primary liver cancers (comprising 90% of all tumors).  

6. **Hepatocellular Failure**

7. **Hepatic Encephalopathy**

   Also called as hepatic coma, is a feature of chronic liver failure.
8. **Portal Hypertension**

In this condition, there is increased resistance to portal blood flow. It may occur in the conditions of Portal vein thrombosis, Splenomegaly, Cirrhosis.

**Types of Liver Dysfunction**

Most of the clinical consequences of liver disease are either as a failure of one of the liver’s four broad functions or as a consequence of portal hypertension, the altered hepatic blood flow of cirrhosis.

**Hepatocyte Dysfunction**

One mechanism of liver disease, particularly in acute liver injury, is dysfunction of the individual hepatocytes that make up the liver parenchyma. The pathway and extent of hepatocellular dysfunction determine the specific manifestations of liver disease. The outcomes to be anticipated when normal hepatic functions fail are described later.

**Portal Hypertension**

Some consequences of liver disease, particularly of cirrhosis, are best understood in terms of what we know about hepatic blood flow. Of greatest clinical importance are the existence under normal circumstances of a low-pressure portal venous capillary bed throughout the liver parenchyma and the functional zonation of portal blood flow.
When pathologic processes (eg, fibrosis) result in elevation of the normally low intrahepatic venous pressure, blood backs up and a substantial fraction of it finds alternative routes back to the systemic circulation, bypassing the liver. Thus, blood from the GI tract is, in effect, filtered less efficiently by the liver before entering the systemic circulation. The consequences of this portal-to-systemic shunting are loss of the protective and clearance functions of the liver, functional abnormalities in renal salt and water homeostasis, and a greatly increased risk of GI hemorrhage from the development of engorged blood vessels carrying venous blood bypassing the liver (esophageal varices).

Even in the absence of any intrinsic parenchymal liver disease, portal to systemic shunting of blood can produce or contribute to encephalopathy (altered mental status resulting from failure to clear poisons absorbed from the GI tract), GI bleeding (resulting from esophageal varices), and malabsorption of fats and fat-soluble vitamins (caused by loss of enterohepatic recirculation of bile), with associated coagulopathy.

**Pathophysiology of Functional Zonation**

The fact that hepatocytes in the different zones of the acinus "see" blood in a particular sequence has great pathophysiologic significance. Because zone 1 hepatocytes see blood that has just left the portal venule or hepatic arteriole, they have access to the highest concentrations of
various substances, both good (eg, oxygen and nutrients) and bad (eg, drugs and toxins absorbed from the GI tract). Zone 2 hepatocytes receive blood containing less of these substances, and zone 3 hepatocytes are bathed in blood largely depleted of them. However, zone 3 hepatocytes see the highest concentrations of products (eg, drug metabolites) released into the bloodstream by hepatocytes of zones 1 and 2. Thus, direct poisons have their most severe impact on zone 1 hepatocytes, whereas poisons that are generated as a result of hepatic metabolism cause more damage to those of zone 3. Similarly, because sinusoidal blood around zone 3 has the lowest oxygen concentration, hepatocytes of this zone are at greatest risk of injury under conditions of hypoxia.

**Manifestations of Liver Dysfunction**

A result of hepatocyte dysfunction or portal-to-systemic shunting, prominent features of liver disease are manifestations of failure of normal functions. An understanding of these mechanisms offers insight into the probable causes of illness in a patient with acute or chronic liver disease.

**Diminished Energy Generation & Substrate Interconversion**

A first category of altered liver function involves the intermediary metabolism of carbohydrates, fats, and proteins.
**Carbohydrate Metabolism**

Severe liver disease can result in either hypoglycemia or hyperglycemia. Hypoglycemia results largely from a decrease in functional hepatocyte mass, whereas hyperglycemia is a result of portal-to-systemic shunting, which decreases the efficiency of postprandial extraction of glucose from portal blood by hepatocytes, thus elevating systemic blood glucose concentration.

**Lipid Metabolism**

Disturbance of lipid metabolism in the liver can result in syndromes of fat accumulation within the liver early in the course of liver injury. Perhaps this is because the complex steps in assembly of lipoprotein particles for export of cholesterol and triglycerides from the liver are more sensitive to disruption than the pathways of lipid synthesis. Such disruption results in a buildup of fat that cannot be exported in the form of VLDL.

In certain chronic liver diseases such as primary biliary cirrhosis, bile flow decreases as a result of destruction of bile ducts. The decrease in bile flow results in decreased lipid clearance via bile, with consequent hyperlipidemia. These patients often develop subcutaneous accumulations of cholesterol termed xanthomas.
Protein Metabolism

Any disturbance of protein metabolism in the liver can result in a syndrome of altered mental status and confusion known as hepatic encephalopathy. As with carbohydrate metabolism, altered protein metabolism can result from either hepatocyte failure or portal-to-systemic shunting, with the net effect of elevation of blood concentrations of centrally acting toxins, including ammonia generated by amino acid metabolism.

Disordered Bile Secretion

The clinical significance of bile synthesis can be seen in the prominence of cholestasis failure to secrete bile in many forms of liver disease. Cholestasis can occur as a result of extrahepatic obstruction (eg, from a gallstone in the common bile duct) or selective dysfunction of the bile synthetic and secretory machinery within the hepatocytes themselves (eg, from a reaction to certain drugs). The mechanisms responsible for cholestatic drug reactions are not well understood. Regardless of the mechanism, however, the clinical consequences of severe cholestasis may be profound: A failure to secrete bile results in a failure to solubilize substances such as dietary lipids and fat-soluble vitamins, resulting in malabsorption and deficiency states, respectively. Retained bile salts are also cytotoxic, but in the setting of cholestasis hepatocytes adapt to decrease uptake of bile salts by down regulating it
while maintaining bile salt excretion. As a result, hepatic necrosis is minimized in predominantly cholestatic syndromes, with the typical laboratory findings of minimally elevated levels of AST and ALT in the presence of marked jaundice and high levels of bilirubin. However, prolonged exposure to bile salts in chronic cholestatic diseases such as primary biliary cirrhosis leads to portal tract cytotoxic injury and inflammation, leading eventually to fibrosis and cirrhosis.

The solubilization function of bile works both to excrete and to absorb substances. Thus, in cholestasis, endogenous substances that are normally excreted via the biliary tract can accumulate to high levels. One such substance is bilirubin, a product of heme degradation. The buildup of bilirubin results in jaundice (icterus), which is a yellow discoloration of the scleras and skin. In the adult, the most significant feature of jaundice is that it serves as a readily monitored index of cholestasis, which may occur alone or with other abnormalities in hepatocyte function (ie, as part of the presentation of acute hepatitis). In the neonate, however, elevated bilirubin concentrations can be toxic to the developing nervous system, producing a syndrome termed kernicterus.

Similarly, cholesterol is normally excreted either by conversion into bile acids or by forming complexes, termed micelles, with preexisting (recycled) bile acids. In cholestasis, the resultant buildup of bile acids
can lead to their deposition in the skin. This is believed to cause intense itching, or pruritus. Data suggest that in at least some patients cholestasis results in altered levels of endogenous opioids. Altered endogenous opioid-mediated neurotransmission may be responsible for pruritus instead of skin deposition of bile acids. Disorders of bile production are a basis for the formation of cholesterol gallstones. Nevertheless, as mentioned, other hepatocyte functions are often relatively well preserved in the face of significant cholestasis.

Impaired Drug Detoxification

Two features of the mechanisms of drug detoxification are of particular clinical importance. One is the phenomenon of enzyme induction. It is observed that the presence in the bloodstream of any of the large class of drugs inactivated by phase I enzymes increases the amount and activity of these enzymes in the liver. This property of enzyme induction makes physiologic sense (as a response to the body's need for increased biotransformation) but can have undesired effects as well: A patient who chronically consumes large amounts of a substance that is metabolized by phase I enzymes (eg, ethanol) will induce high levels of these enzymes and thus speed up the metabolism of other substances metabolized by the same detoxifying enzymes (eg, antiseizure or anticoagulant medications, resulting in subtherapeutic blood levels of the drugs).
A second clinically important phenomenon in drug metabolism is, phase I reactions, often convert relatively benign compounds into more reactive and hence more toxic ones. Normally, this heightened reactivity of phase I reaction products serves to facilitate phase II reactions, making detoxification more efficient. However, under certain conditions when phase II reactions are impaired (eg, during glutathione deficiency from inadequate nutrition), continued phase I enzyme activity can cause increased liver injury. This is because the products of many phase I reactions, in the absence of glutathione, react with and damage cellular components. Such damage rapidly kills the hepatocyte.

Thus, the combined effects of certain common conditions can make the individual abnormally sensitive to the toxic effects of drugs. For example, the combination of induced phase I activity (eg, caused by alcoholism) with low phase II activity (eg, caused by low glutathione levels from nutritional deprivation) can result in heightened generation of reactive intermediates with an inadequate capacity to conjugate and detoxify them. A classic example of this phenomenon is acetaminophen toxicity. As little as 2.5 g of acetaminophen can produce significant damage in such susceptible individuals, whereas normal individuals have the capacity to detoxify 10 g/d or more.


**Lipoprotein Dynamics and Dyslipidemias**

The liver’s role in lipid metabolism is illustrated by the genetic defect causing familial hypercholesterolemia. Lack of a functional LDL receptor in such cases renders the liver unable to clear LDL cholesterol from the bloodstream, resulting in markedly elevated serum cholesterol and accelerated atherosclerosis and coronary artery disease. Heterozygotes with one normal LDL receptor allele can be treated with drugs (eg, HMG-CoA reductase inhibitors) that inhibit endogenous cholesterol synthesis and thus upregulate LDL receptor levels. However, there is no effective drug therapy for homozygotes because they have no normal LDL receptors. Hepatic transplantation is effective therapy for homozygous familial hypercholesterolemia because it provides a genetically different liver with normal LDL receptors.

In acquired liver diseases, the serum cholesterol is elevated in biliary tract obstruction as a result of blockage of cholesterol excretion in bile, and it is diminished in severe alcoholic cirrhosis, in which fat malabsorption prevents cholesterol intake.

**Altered Hepatic Binding and Storage Functions**

Liver disease influences the liver’s ability to store various substances. As a result, patients with liver disease are at high risk for certain deficiency states such as folic acid and vitamin B12 deficiency.
Because these vitamins are needed for DNA synthesis, their deficiency results in macrocytic anemia (low red blood cell count with large red cells reflecting abnormal nuclear maturation), a common finding in patients with liver disease.

**Diminished Synthesis & Secretion of Plasma Proteins**

The clinical significance of liver protein synthesis and secretion derives from the wide range of functions carried out by these proteins. For example, because albumin is the major contributor to plasma oncotic pressure, hypoalbuminemia as a consequence of liver disease or nutritional deficiency presents with marked edema formation. Other important proteins synthesized and secreted by the liver include clotting factors and hormone-binding proteins.

**Loss of Protective & Clearance Functions**

A crucial protective function of the liver is its role as a filter of blood from the GI tract, by which various substances are removed from portal blood before it reenters the systemic circulation.

**Clearance of Bacteria and Endotoxin**

Clearance of bacteria by Kupffer cells of the liver is the final line of defense in keeping gut-derived bacteria out of the systemic circulation. Loss of this capacity in liver disease as a result of portal-to-systemic
shunting may help to explain why, in patients with severe liver disease, infections can rapidly become systemic and result in sepsis.

**Altered Ammonia Metabolism**

Impairment of the liver’s ability to detoxify ammonia to urea leads to hepatic encephalopathy, manifested as an altered mental status. This may be an early manifestation of acute fulminant hepatitis with massive hepatocellular dysfunction even before the development of maximal hepatocellular necrosis. It can be a final step in progressive chronic liver disease with diminished hepatocyte functional capacity.

Most often it is a consequence of an increased ammonia load in a patient with marginal liver function or significant portal-to-systemic shunting. Thus, encephalopathy may occur as a first sign of renewed GI bleeding (as a result of increased production of ammonia and other products caused by breakdown of blood protein by GI tract microbes) or may simply be due to increased protein intake (eg, a cheeseburger eaten by a patient with cirrhosis). Finally, the development of sepsis in these patients results in increased endogenous protein catabolism and, therefore, elevated ammonia production in the face of a decreased capacity for ammonia detoxification because of the liver disease. Thus, the development of encephalopathy in a patient with chronic liver disease calls for investigation of possible acute GI bleeding as well as potentially catastrophic infection. Pending the outcome of diagnostic studies (eg,
serial hematocrit measurements and cultures of blood, urine, and ascitic fluid), therapy is designed to improve mental status by diminishing the absorption of ammonia and other noxious substances from the GI tract. When the patient is given the nonabsorbable carbohydrate lactulose, whose metabolism by microbes creates an acidic environment, ammonia is trapped as the charged NH4+ species in the gut lumen and excreted by the resultant osmotic diarrhea. Thus, this toxin is prevented from ever entering the portal circulation, and the patient’s mental status gradually improves. Lactulose also selects for a gut bacterial flora that produces less ammonia.

Furthermore, the resulting elevated blood ammonia and other nitrogen containing compounds can upregulate peripheral receptors for endogenous benzodiazepine-like products. These effects may contribute to altered systemic hemodynamics in liver disease.

**Altered Hormone Clearance in Liver Disease**

Normally, the liver removes from the bloodstream the fraction of steroid hormones not bound to steroid hormone-binding globulin. On uptake by hepatocytes, these steroids are oxidized, conjugated, and excreted into bile, where a fraction undergoes enterohepatic circulation. In liver disease accompanied by significant portal-to-systemic shunting, steroid hormone clearance is diminished, extraction of the enterohepatic circulated fraction is impaired, and enzymatic conversion
of androgens to estrogens (peripheral aromatization) is increased. The net effect is an elevation of blood estrogens, which in turn alters hepatocyte protein synthesis as well as secretion and microsomal P450 activity. Synthesis of some hepatic proteins increases, whereas synthesis of others is diminished. P450 activity, increases, as the liver attempts to partially compensate for the higher blood estrogen levels by increased metabolism. Thus, male patients with liver disease display both gonadal and pituitary suppression as well as feminization.

**Sodium & Water Balance**

Patients with liver disease often display renal abnormalities and complications, most commonly sodium retention and difficulty excreting water. An intrinsic renal lesion is apparently not involved, because the kidneys of patients with liver disease typically function normally when transplanted into patients whose liver is normal. Instead, renal abnormalities associated with liver disease are functional, occurring because liver disease induces altered intravascular pressures and perhaps because of elevated nitric oxide levels or loss of as yet poorly understood factors secreted from the liver or the endothelium. By whatever homeostatic mechanisms, intravascular volume is perceived as being inadequate when it is actually only maldistributed. Renal mechanisms of salt and water retention are then stimulated to correct what has been sensed as volume depletion.
Causes of liver disease:

In many cases the dysfunction of liver will be secondary to a problem elsewhere in the body.

**Trauma:** Animals that receive a severe and blunt blow on the abdomen can suffer from liver disease. The most common cause of this type of blow is being hit by a car there by a liver lobe fracture and bleeding into the abdomen, even leading to death and more common occurrence is a bruise (contusion). Heat stroke, diaphragmatic hernia and liver lobe torsion can also cause liver problems.

**Inflammation:** An inflamed condition of liver is called hepatitis. Trauma can cause this, along with other drugs, viruses, bacteria, bile and toxins.

**Pancreatitis:** The severe inflammatory process that occurs with digestive enzymes can spill over into the liver and cause severe disease.

**Anemia:** Hemolytic anemia can decrease the oxygen availability to liver cells and lead to their death.

**Infection:** Bacteria, viruses, and fungi can all cause liver disease. Since bacterial infection is common in many liver problems it is routine to use antibiotics when treating liver problems. Specific diseases include infectious canine hepatitis, canine herpes virus, feline infectious
peritonitis (FIP), leptospirosis, abscesses, histoplasmosis, coccidiomycosis and toxoplasmosis. Acute viral hepatitis is a systemic infection manifested primarily by an acute attack on the hepatocytes. Five hepatotropic viruses have been identified (HAV, HBV, HCV, HDV, HEV). Hepatitis A (HAV) causes acute, self-limited disease that is transmitted orally. Hepatitis B (HBV) and hepatitis C viruses (HCV) are transmitted by exchange of body fluids such as blood transfusion or sexual contacts. Hepatitis D (HDV) is a viroid that causes inflammation only in concrete with HBV. Hepatitis E (HEV) virus is transmitted by enteric route and cause self limited diseases.

Chronic hepatitis is an uncommon, but important, complication of HBV- HDV infection. The liver injury also results from an inflammatory immune attack against hepatocytes. In drug-induced hepatitis, a number of drugs have been reported, including methyldopa, nitrofurantoin, isoniazid, ketoconazole and acetaminophen.

**Toxins:** There are literally thousands of chemicals that could be toxic to the liver and a few examples of these chemicals that are commonly used in the treatment include:

- Rimadyl (arthritis treatment) in Labradors
- Thiacetarsamide (heartworm treatment)
- Ketoconazole (fungal treatment)
Tylenol (acetaminophen)

Glucocorticoids (cortisone)

Anthelmintics (deworming medication)

Parasiticides

**Cancer:** Cancer can arise directly within the liver (primary) or spread from elsewhere (metastatic or secondary) through the circulatory or lymphatic systems. In the anatomy section as mentioned the dual blood supply to the liver; the portal vein and the hepatic artery. This extra blood supply increases the chance that a tumor in a different organ that has spread into the bloodstream will end up in the liver. As mentioned in the physiology section, liver cancer is usually detected only after the disease is well established, since functional reserve capacity allowed the liver to function normally for a prolonged period of time.

**Some of these liver cancers include:**

Primary:

- Lymphosarcoma

- Hemangiosarcoma

- Metastatic:

- Adenocarcinoma
- Mammary tumors
- Oral carcinoma
- Lymphosarcoma
- Hemangiosarcoma

**Metabolic diseases that cause secondary liver problems:**

- Hypothyroidism
- Hyperthyroidism
- Pancreatitis
- Diabetes mellitus
- Cushing's syndrome
- Inflammatory bowel disease
- Hypoadrenocorticism
- Protein-losing enteropathy
Table 6: Clinical Consequences of Liver Disease

| Characteristic signs                          | Hepatic dysfunction :                   |
|                                             | Jaundice and cholestasis                |
|                                             | Hypoalbuminemia                         |
|                                             | Hyperammonemia                          |
|                                             | Hypoglycemia                            |
|                                             | Fetal hepaticus                         |
|                                             | Palmar erythema                         |
|                                             | Spider angiomas                         |
|                                             | Hypogonadism                            |
|                                             | Gynecomastia                            |
|                                             | Weight loss                             |
|                                             | Muscle wasting                          |
|                                             | Portal hypertension from cirrhosis :    |
|                                             | Ascites                                 |
|                                             | Splenomegaly                            |
|                                             | Hemorrhoids                             |
|                                             | Caput medusae-abdominal skin.           |

| Life-threatening complications               | Hepatic failure                         |
|                                             | Multiple organ failure                   |
|                                             | Coagulopathy                            |
|                                             | Hepatic encephalopathy                   |
|                                             | Hepatorenal syndrome                     |
|                                             | Portal hypertension from cirrhosis :     |
|                                             | Malignancy with chronic disease          |
|                                             | Hepatocellular carcinoma                 |
### Table 7: Laboratory Evaluation of Liver Disease

<table>
<thead>
<tr>
<th>Test category</th>
<th>Serum measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hepatocyte integrity</td>
<td>Cytosolic hepatocellular enzymes</td>
</tr>
<tr>
<td></td>
<td><em>Serum aspartate aminotransferase (AST)</em></td>
</tr>
<tr>
<td></td>
<td><em>Serum alanine aminotransferase (ALT)</em></td>
</tr>
<tr>
<td></td>
<td>Serum lactate dehydrogenase (LDH)*</td>
</tr>
<tr>
<td>Biliary excretory function</td>
<td>Substances normally secreted in the Serum bilirubin</td>
</tr>
<tr>
<td></td>
<td><em>Total</em>: unconjugated plus conjugated</td>
</tr>
<tr>
<td></td>
<td><em>Direct</em>: conjugated only</td>
</tr>
<tr>
<td></td>
<td>Delta: covalently linked to albumin *</td>
</tr>
<tr>
<td></td>
<td><em>Urine bilirubin</em></td>
</tr>
<tr>
<td></td>
<td>Serum bile acids*</td>
</tr>
<tr>
<td></td>
<td>Plasma membrane enzymes</td>
</tr>
<tr>
<td></td>
<td>(from damage to bile canaliculus)</td>
</tr>
<tr>
<td></td>
<td><em>Serum alkaline phosphatase</em></td>
</tr>
<tr>
<td></td>
<td>Serum γ-glutamyl transpeptidase*</td>
</tr>
<tr>
<td></td>
<td>Serum 5'-nucleotidase*</td>
</tr>
<tr>
<td>Hepatocyte function</td>
<td>Proteins secreted in to the blood Serum albumin+</td>
</tr>
<tr>
<td></td>
<td>Prothrombin time* (factors V, VII, X, prothrombin, fibrinogen)</td>
</tr>
<tr>
<td></td>
<td>Hepatocyte metabolism</td>
</tr>
<tr>
<td></td>
<td>Serum ammonia*</td>
</tr>
<tr>
<td></td>
<td>Aminopyrine breath test (hepatic demethylation)+</td>
</tr>
<tr>
<td></td>
<td>Galactose elimination (intravenous injection)+</td>
</tr>
</tbody>
</table>
The most common tests are in italics,*An elevation implicated liver disease,+A decrease implicates liver disease.

### Table 8: Types of Hepatotoxic Agents

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INORGANIC AGENTS</strong></td>
<td>Metals and metalloids: antimony, arsenic, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, gold, phosphorous, selenium, tellurium, thallium, zinc, hydrazine derivatives, iodides.</td>
</tr>
<tr>
<td><strong>ORGANIC AGENTS</strong></td>
<td></td>
</tr>
<tr>
<td>Natural :</td>
<td></td>
</tr>
<tr>
<td>Plant toxins</td>
<td>Albitocin, cycasin, nutmeg, tannic acid, icterogenin, pyrrolidizines, saferole, indospicine.</td>
</tr>
<tr>
<td>Mycotoxins</td>
<td>Aflatoxins, cyclochlorotine, ethanol, luteoskyrin, griseofulvin, sporidesmin, tetracycline, and other antibiotics.</td>
</tr>
<tr>
<td>Bacterial toxins</td>
<td>Exotoxins (<em>C. diphteria, Clostridium botulinus</em>), endotoxins, ethionine.</td>
</tr>
<tr>
<td>Synthetic:Non-medicinal</td>
<td>Haloalkanes and haloolephins, Nitroalkanes, Chloroaromatic compounds, Nitroaromatic compounds, organic amines, Azo compounds. Phenol and derivatives, various other organic compounds.</td>
</tr>
<tr>
<td><strong>MEDICINAL AGENTS</strong></td>
<td></td>
</tr>
<tr>
<td>Category of drugs</td>
<td>Examples</td>
</tr>
<tr>
<td>Neuro psychotropics</td>
<td>Hydrazine, tranylcypromine anticonvulsants,</td>
</tr>
<tr>
<td>Category of agents</td>
<td>Mechanism of action</td>
</tr>
<tr>
<td>--------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td><strong>Intrinsic Toxicity</strong></td>
<td></td>
</tr>
<tr>
<td>Direct</td>
<td>Direct Physicochemical destruction by peroxidation of hepatocytes.</td>
</tr>
<tr>
<td>Indirect cytotoxic</td>
<td>Interference with hepatocellular metabolic pathways</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>Interference with bile destruction</td>
</tr>
<tr>
<td>Host Idiosyncracy</td>
<td>Hypersensitivity</td>
</tr>
<tr>
<td>------------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Production of hepatotoxic metabolites</td>
</tr>
</tbody>
</table>

2.7.1 *In vivo* Models for hepatoprotectives

A toxic dose or repeated doses of a known hepatotoxin (paracetamol, carbon tetrachloride, thioacetamide, alcohol, D-Galactosamine/lipopolysaccharide, azathioprine, tert – butyl hydroperoxide, allylalcohol, etc) are administrated, to induce liver damage in experimental animals. The test substance is administered along with, prior to and/or after the toxin treatment. If the hepatotoxicity is prevented or reduced by the pre-treatment or after toxin challenge then it is inferred that the test substance is effective\(^\text{82-88}\).

Liver damage and recovery from damage are assessed by measuring serum marker enzymes, bilirubin, histopathological changes in the liver, biochemical changes in liver (e.g.: hydroxyproline, lipid etc) and bile flow. When the liver is damaged, liver-enzymes such as glutamate pyruvate transaminase (GPT), glutamate oxaloacetate
transaminase (GOT) and alkaline phosphatase enter into the circulation. An increase in the levels of these marker enzymes in the serum is an indication of liver damage. Other effects of induced liver damage such as reduction of prothrombin synthesis giving an extended prothrombin time and reduction in clearance of certain substances such as bromosulphthalein can be used in the evaluation of hepatoprotective plants.

The hepatoprotective effect of a drug against different hepatotoxins differs especially when the mechanism of action of toxins are different. Therefore, the efficacy of each drug has to be tested against hepatotoxins, which act by different mechanisms.

**Mechanism of D-Galactosamine (D-GalN) / Lipopolysaccharide (LPS) induced hepatotoxicity**

D-GalN/LPS induced hepaotocelellar damage, a well-established model of hepatitis takes advantage of the ability of D-GalN to potentiate the toxic effects of LPS producing fulminant hepatitis within a few hours of administration. A high dose of D-GalN causes necrosis of the liver by UTP depletion and inhibition of protein synthesis, although D-GalN is often used in combination with lipopolysaccharide or tumor necrosis factor. Accumulation of UDP-sugar nucleotides may contribute to the changes in the rough endoplasmic reticulum and to the disturbance in the protein metabolism. Further, intense galactosamination of membrane
structure is thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium pump, with consequent increase in the intracellular calcium is considered to be responsible for cell death. In recent years, apart from the well documented inhibition of protein synthesis, it has been suggested that reactive oxygen species produced by activated macrophages might be the primary cause in D-GalN-induced liver damage. Liver damage induced by D-GalN/LPS generally reflects disturbances of liver cell metabolism which lead to characteristic changes in the activities of serum enzymes. The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membranes. In this context, we can observe a significant increase in the serum activities of AST, ALT, ALP, LDH and γ-GT which is in accordance with the earlier findings. Because the levels of these marker enzymes are proportional to the extent of damage, the activity of these enzymes can be used for diagnosis as indicators of prognosis of the disease.

The rise in serum levels of AST and ALT has been attributed to the damaged structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage.

**Ethyl alcohol induced hepatotoxicity**

Alcoholic liver disease continues to the most serious liver disorder throughout the world, including India, where alcohol is mainly
consumed in the form of country made liquor (CML). Alteration in liver functions due to alcohol range from fatty liver to cirrhosis. After its ingestion, ethanol is readily absorbed from the gastrointestinal tract. Only 2-10% of that absorbed is eliminated through the kidneys and lungs; the rest is oxidized in the body, mainly in the liver.

The hepatocytes contains three main pathways for ethanol metabolism, each located in a different sub cellular compartment: the alcohol dehydrogenase (ADH) pathway of the cytosol, the microsomal ethanol oxidizing system (MEOS) located in the endoplasmic reticulum and catalase located in the peroxisomes.

**Figure 10: Metabolic Pathway of Alcohol**
**Alcohol Dehydrogenase Pathway**

A major pathway for ethanol disposition involves ADH, an enzyme that catalyzes the conversion of ethanol to acetaldehyde. In ADH-mediated oxidation of ethanol, hydrogen is transferred from the substrate to the cofactor nicotinamide adenine dinucleotide (NAD), converting it to its reduced form (NADH) and acetaldehyde is produced. The dissociation of the NADH-enzyme complex has been shown to be a rate limiting step in this reaction. As a net result, the first step in the oxidation of ethanol generates an excess of reducing equivalents in the cytosol, primarily as NADH. In normal rates when ethanol is given there is marked shift redox potential of the cytosol as measured by changes in the lactate: pyruvate ratio that leads to hyperlactacidemia because of both decreased utilization and enhanced production of lactate by the liver.

The altered redox state also impairs gluconeogenesis from amino acids and favors hypoglycemia. The increased NADH/NAD ratio raises the concentration of glycerophosphate, which favors hepatic triglyceride accumulation by trapping fatty acids. A major interaction site of ethanol in the citric acid cycle (in the mitochondria) is found to be with ketoglutarate oxidation. Moreover, the redox change associated with ethanol oxidation decreases the hepatic concentration of oxaloacetate, the availability of which controls the activity of citrate synthetase. The mitochondria will therefore use the hydrogen equivalents originating
from ethanol, rather than from oxidation through the citric acid cycle of two carbon fragments derived from fatty acids. Thus, fatty acids that normally serve as the main energy source for the liver are supplanted by ethanol. Depressed fatty acid oxidation by ethanol has been demonstrated in liver slices, isolated hepatocytes, human liver biopsy tissue and in vivo. This change results in the deposition of fat in the liver, the first stage of alcoholic liver injury. In other experimental model, chronic alcohol consumption is associated with the progression of alcoholic liver injury beyond the fatty liver stage, affecting even protein metabolism.

The capacity of acetaldehyde to cause lipid peroxidation has been demonstrated in isolated perfused livers and has been linked to acetaldehyde oxidation. In addition, the binding of acetaldehyde with cysteine, cysteine containing glutathione or both may contribute to the depression of liver glutathione, thereby reducing the scavenging of toxic free radicals by this tripeptide. Rats given ethanol for long periods have significantly increased rates of glutathione turnover in association with increased activity of hepatic gamma-glutamyl transpeptidase. Severe glutathione reduction favours lipid peroxidation which can be prevented or impaired in vivo by the administration of methionine, a precursor of cysteine and glutathione. The increased activity of microsomal NADPH oxidase after ethanol consumption may result in enhanced superoxide
and hydrogen peroxide production, thereby theoretically favouring lipid peroxidation.

In addition, it has been postulated that purine metabolism by means of xanthine oxidase may lead to the production of oxygen radicals. Another potential mechanism of cellular injury in acute alcoholic liver disease is generation of free-radicals by neutrophils\textsuperscript{71}.

Although the pathogenesis of early alcoholic liver disease is still largely unknown, accumulating evidence suggests that endotoxins (lipopolysaccharide (LPS), tumor necrosis factor α (TNF-α) and free radicals are involved. Ethanol increases permeability of the isolated small bowel to endotoxin and elevates circulating endotoxin. This is most likely the starting point of a pathophysiologic cascade leading to liver injury. Circulating LPS associates with LPS-binding protein (LBP) and the LPS-LBP complex binds to the CD14 receptor of Kupffer cell, the resident liver macrophages are the major population of the monocyte macrophage lineage. Recently, early alcohol induced liver injury was blocked in CD14 and TLR-4 knock out mice. The interaction of LPC with CD14 triggers a signaling cascade and activates kuffer cells that release many potent effectors cytokines. Furthermore, in humans, it was recently shown that promoter polymorphism of the CD14 receptor gene is a risk factor for alcoholic liver disease.
Recently, a study with the continuous intragastric feeding model in mice showed that early alcohol-induced liver injury was blocked in animals lacking TNF-α in bile from rat exposed to ethanol in the Tsukamoto-French model using the spin-trapping technique and Electron Spin Resonance (ESR) spectroscopy. Free radical signals were diminished over 50% when kupffer cells were destroyed by treatment with gadolinium chloride (GdCl₃).

**Microsomal ethanol oxidizing system (MEOS)**

The first indication of an interaction of ethanol with the microsomal fraction of the hepatocyte was provided by the morphologic observation that in rats, ethanol feeding results in a proliferation of the smooth endoplasmic reticulum (SER), this resembles the change seen after the administration of many xenobiotics compounds including known hepatotoxins, numerous therapeutic agents and food additives. Most of the substances that stimulate proliferation of the SER are metabolized, at least in part, in the microsomal fraction of the hepatocyte. This observation raised the possibility that, in addition to its oxidation by ADH in the cytosol, ethanol may be metabolized by the microsomes. The system required NADPH & O₂ and was relatively insensitive to catalase inhibition. Furthermore, the MEOS was differentiated from catalase by it’s to oxidase long – chain aliphatic alcohols, which are not substrate for catalase⁷⁰.
Role of catalase:

Catalase is a haemoprotein located in the peroxisomes of most tissues. Small amounts are also found in isolated hepatocyte microsomes. As early as 1936 Keilin and Hartee suggested that catalase may play a role in alcohol metabolism. In 1955 this was confirmed by Laser who showed that ethanol could be effectively oxidized in the presence of hydrogen peroxide and catalase.

Catalase of oxidizing ethanol in-vitro only in the presence of a hydrogen peroxide generating system. The reaction is limited by the rate of hydrogen peroxide generation rather than by the amount of catalase itself. The physiological rate of hydrogen peroxide production is less, suggesting that catalase could account for only 2% of the in-vivo rate of ethanol oxidation.

Mechanism of carbon tetrachloride (CCl₄) induced hepatotoxicity:

CCl₄ is a potent hepatotoxin producing centrilobular hepatic necrosis, which causes liver injury.

CCl₄ induces fatty liver and cell necrosis and play a significant role in inducing triacylglycerol accumulation, depletion of GSH, increased lipid peroxidation, membrane damage, and depression of protein synthesis and loss of enzyme activity. Being cytoplasmic in location the damage marker enzymes GOT, GPT and HDL are released in the serum.
It is now generally accepted that the hepatotoxicity of CCl₄ is the result of reductive dehalogenation, which is catalyzed by cytochrome P450 enzyme and forms the highly reactive trichloromethyl free radical. This then readily interacts with molecular oxygen to form the trichloromethyl peroxy radical. The free radical can form covalent bond with sulfahydryl group, such as glutathione (GSH), protein thiol and lipids or abstracting a hydrogen atom from an unsaturated lipid. This covalent binding of free radical to cell macromolecules is considered the initial step in a chain of events, which eventually leads to membrane lipid peroxidation, liver damage and finally cell necrosis.

CCl₄ is reductively converted by P450 to the trichloromethyl radical the fate of this radical is of interest. First the radical add covalently to unsaturated fatty acids, trichloromethyl fatty acids, particularly of membrane phospholipids.

Figure 11: Schematic Representation of Reactive Mechanism of CCl₄ Induced Hepatic Injury
Recently these substituted fatty acids have been noted to be partially resistant to replace from endoplasmic reticular phospholipase A₂. This seems to be result of cross linking of trichloromethyl fatty acid radical, which adds to double bond of another adjacent fatty acids (link).

![Figure 12: Covalent Binding to Lipids](image)

The physiologic significance of this cross-linking on membrane structure and function may be of great importance, particularly if these phospholipids are transformed to other critical sites in the cell. Besides covalent binding to lipid, the cells can abstract an electron from unsaturated fatty acids, yielding CHCl₃ and or fatty acid radical. Either the trichloromethyl fatty acid radical or the fatty acid radical can react with oxygen to form peroxy radical, which initiates the lipid peroxidation chain reaction.

**Mechanism of paracetamol (PCM) induced hepatotoxicity**

Paracetamol (N-acetyl-p-aminophenol) is a widely used analgesic and antipyretic drug and is safe when used in therapeutic doses. However, over dosage of paracetamol is known to be hepatotoxic and nephrotoxic in man and in experimental animals⁸². Paracetamol is a
direct hepatotoxin i.e. intoxication is dose dependent and reproducible\textsuperscript{83}. Exposure of animals to higher doses produces centrilobular or massive hepatic necrosis followed by congestion and failure. The hepatic necrosis is associated with damage to sub cellular organelle including mitochondria. Thus the drug is used as a typical hepatotoxin to produce hepatic failure experimentally.

At lower doses, about 80\% of ingested paracetamol is eliminated mainly as sulfate and glucuronide conjugates before oxidation and only 5\% is oxidized by hepatic cytochrome P450 (CYP2E\textsubscript{1}) to a highly reactive and toxic electrophile i.e. N-acetyl-p-benzoquinemine (NAPQI). After over dosage of paracetamol the glucoronidation and sulfation routes become saturated and as a consequence, paracetamol is increasingly metabolized into NAPQI. Semiquinone radical, one-electron reduction metabolite of NAPQI mediates the cytotoxic effects of NAPQI. Production of these toxic semiquinone radicals is catalyzed by the microsomal cytochrome P450 reductase. These semiquinone radicals, in turn, can bind directly with cellular macromolecules to produce toxicity or alternatively, the radical can be reoxidized back to their original quinones by donating one electron to molecular oxygen under aerobic conditions. This donation of one electron then generates reduced oxygen radical species and hydroxyl radical. Both semiquinone and oxygen radical are known to be responsible for cytotoxic effects observed with quinones.
Alternatively to this toxic one-electron reduction pathway quinone compounds also can be reduced by a direct two-electron reduction pathway to non-toxic hydroquinones, either enzymatically or by quinone reduction of two molecules of GSH. Both of these direct two-electron reductions will occur without any production of the toxic semiquinone or oxygen radicals and therefore, may provide a competitive protection pathway against the toxicity caused by one-electron reduction of NAPQI. Also NAPQI is detoxified by glutathione (GSH) to form 3-(GSH-S-yl) acetaminophen. Paracetamol overdose saturates the nontoxic metabolic pathway, i.e. sulfation, glucuronidation, and detoxification of NAPQI by glutathione. The reactive NAPQI may oxidize and arylate cysteiny1 thiol group, forming adducts which inhibit the function of cellular proteins. Adducts formation has been demonstrated for a selenium-binding protein, for microsomal subunit of glutamine. Other mechanism, such as oxidation of pyridine nucleotides and lipid peroxidation, may contribute to cell damage by paracetamol overdose.

Nevertheless at high doses of paracetamol NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which result in the liver GSH pool depletion and the reactive intermediate reacts with other nucleophilic centers of vital molecules in liver cells leading subsequently to hepatotoxicity. Besides, paracetamol is also shown to directly inhibit cellular proliferation, induce oxidative stress, resulting in lipid
peroxidation, deplete ATP levels and alter Ca\textsuperscript{++} homeostasis; all of these changes are considered potentially fatal to the cell.

**Figure 13: Metabolic Pathway of Paracetamol (acetaminophen)**

**Mechanism of Thioacetamide Induced Hepatotoxicity:**

Thioacetamide was originally used as a fungicide to protect against decay of organs. It was soon recognized as a potent hepatotoxin and carcinogen in rat. The compound has also been reported to be toxic to kidney and thymus. It is also reported that chronic thioacetamide exposure produces cirrhosis in rat. Its long term administration causes the development of cirrhosis associated with an increased extent of lipid
peroxidation. The toxicity experienced by the liver during thioacetamide poisoning results from the production of its metabolite, namely thioacetamide-5-oxide, which is a direct hepatotoxin. Thioacetamide is metabolised by liver CYP 450 2E1, enzymes, rendering sulfone and sulfoxide derivatives which are apparently responsible for structural proteins and enzyme inactivation.

The thioacetamide-5-oxide is responsible for the change in cell permeability, increased intracellular concentration of calcium, increase in nuclear volume and enlargement of nucleoli and also inhibits mitochondrial activity which leads to cell death.

Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver. Damage to liver cell causes leakage of cellular enzyme into serum.

*In-vivo* administration of thioacetamide to rodents results in cell death in centrilobular zones both by apoptosis and necrosis. The cellular changes induced by apoptosis occur after a cascade of cell signaling and cascade mediated events and is triggered by two major pathways: extrinsic and intrinsic pathway.

The extrinsic pathway implicates death ligands such as Fas ligand, TNFα, TRAIL and their receptors. The intrinsic pathway includes apoptotic stimuli induced by cytotoxic drugs or oxidative stress which
target mitochondria. This pathway involves the release of cytochrome C from mitochondria to the cytosol, which induces apoptosome complex formation and results in protease pro-caspase-9 activation and subsequent activation of pro-caspase-3 through proteolytic cleavage visualized by the decrease of proform level and appearance of cleavage products. Both the pathway leads to caspase-3 activation and cleavage of limited set of essential cellular protein, leading to cell dismantlement. In the liver the apoptosis induced by thioacetamide could result from a combination of both pathways: intrinsic apoptosis pathway by generation of oxidative stress and the extrinsic apoptosis pathway by activation of kupffer cells which can secrete TNFα.

**Mechanism of azathioprine (AZP) induced hepatotoxicity:**

The AZP-induced hepatotoxicity observed 24 hours post treatment is documented by significant increments in the activities of both serum ALT and AST and confirmed by histological changes in liver of male albino rats. Most of the hepatocytes in AZP-treated rats displayed mild cellular degeneration and loss of their characteristic configuration. Moreover, the marked necrobiotic changes in the liver were mainly in the form of degenerated, vacuolated cells and karyolysis or pyknosis of nuclei. Histopathological changes in the liver included also dilatation of blood vessels, congestion in the lobules, some hemorrhagic coagulative foci in hepatic parenchyma and infiltration of mixed inflammatory cells
around the necrotic hepatocytes. In this report, the AZP-induced hepatotoxicity. Mammalian model was successfully established as reflected in the dramatic increase of the liver function indictors (ALT, AST). A single dose of AZP has been shown to increase the serum ALT and AST activities 24 hours post treatment. Interestingly, levels of the endogenous liver antioxidants such as GSH, CAT and SOD enzymes as well as the MDA showed clear association with the developed AZP-induced heptotoxicity. In hepatocytes GSH is consumed during metabolism of AZP to 6-mercaptopurine (6-MP). The mechanism of AZP toxicity to hepatocytes involves depletion of GSH leading to mitochondrial injury with profound depletion of ATP and cell death by necrosis. Lipid peroxidation is a free radical-inducible process in which membrane polyunsaturated fatty acids are oxidative degraded into a variety of products including MDA. Therefore it is conceivable that AZP-induced depletion of hepatic GSH and its associated increase of MDA originated as a result of the AZP-induced elevation of free radicals which in turn speed up lipid peroxidation and cause irreversible cell damage.

**Mechanism of tert-Butyl alcohol (t-BHP) induced hepatotoxicity:**

*t*-BHP is metabolized in hepatocytes by two distinct pathways. One involves cytochrome P450, leading to the formation of toxic peroxyl and alkoxy radicals that initiate lipid peroxidation, affect cell integrity and form covalent bondswith cellular molecules, resulting in cell death. The
second toxicological pathway of t-BHP is a detoxification reaction involving glutathione peroxidase, which gives rise to t-butanol and GSSG that in turn alters Ca2+ homeostasis and increases physiological formation of ROS. GSH is widely distributed among living cells and is involved in many biological functions. It is well-established that GSH acts as an essential intracellular reducing agent for maintenance of antioxidant molecules and the thiol groups on intracellular proteins, namely de Ca2+-ATPase transporter of endoplasmic reticulum. GSH is also the most important biomolecule protecting against chemically induced cytotoxicity, by participating in the elimination of reactive intermediates by conjugation and hydroperoxide reduction, or of free radicals by direct quenching. t-BHP caused a significant depletion in total glutathione and GSH contents. An important aspect of t-BHP hepatotoxicity is related with its reduction by glutathione peroxidase to the corresponding alcohol, at the expense of GSH which is converted to GSSG. Under severe exposure to t-BHP, the reduction of GSSG by glutathione reductase or the regeneration of NADPH may be insufficient, leading to GSSG accumulation. Metabolisation of t-BHP mediated by cytochrome P450 is an additional factor for the depletion of GSH, since it scavenge the resulting peroxyl and alkoxy radicals being oxidised to GSSG. GSH depletion was not proportionally correlated with GSSG increase and these results can be due to covalent binding of GSH to some electrophilic species generated from t-BHP metabolism or to GSH reaction with
protein thiols, protecting them from oxidation. Lipid peroxidation has been recognized as a potential mechanism of cell injury\textsuperscript{97}.

**In vitro Studies:**

Fresh hepatocyte preparations and primary cultured hepatocytes are used to study direct anti-hepatotoxic activity of drugs. Hepatocytes are treated with hepatotoxin and the effect of the plant drug on the same is evaluated. The activities of the transaminases released into the medium are determined. An increase in the activities in the medium indicated liver damage. Parameters such as hepatocyte multiplication, morphology, macromolecular synthesis and oxygen consumption are determined.

**Biochemical Assays:**

Since, many toxic chemicals induce liver damage by inducing lipid peroxidation and/or oxidative damage to DNA and reduction in the levels of glutathione, assessment of antioxidant property is useful. Antioxidant property of plant drugs is studied using liver homogenates, isolated liver cell membranes, DNA etc. In the process leading to cirrhosis, accumulation of connective tissue and parenchymal regeneration are competing events. Therefore, the search for agents to prevent liver cirrhosis is also focused on inhibitors of excessive connective tissue
formation in the liver. Fibro-suppressive effects by inhibitors of protein hydroxylation can be screened.

**Free Radical Generated Radical Hepatotoxicity:**

The presence of free radicals in biological materials was discovered less than 50 years ago. Denham Harman hypothesized that oxygen radical may be formed as by-products of enzymatic reaction in vivo. He described that free radical may account for gross cellular damage, mutagenesis, and cancer and last but not least, the degenerative process of biological aging.

**Major Types of Free Radicals:**

**Reactive oxygen species (ROS):**

The superoxide anion is formed by the univalent reduction of triplet-state molecular oxygen (O$_2$).

This process is medicated by enzymes such as NAD (P) H oxidases xanthine oxidase. Superoxide dismutase (SOD) converts superoxide enzymatically in to hydrogen peroxide. In the presence of reduced transition metals (ex. Ferrous or cuprous ions), hydrogen peroxide can be converted in to highly reactive hydroxyl radical (OH$^-$. Alternatively hydrogen peroxide may be converted in to water by the enzyme catalase or glutathione peroxidase. In glutathione peroxidase reaction glutathione
is oxidized to glutathione by glutathione reductase in an NADPH-consuming process.

**Figure 14: Pathway of Reactive Oxygen Species (ROS) Production and Clearance**

**Reactive nitrogen species (RNS):**

The nitrogen radical (NO⁻) is produced in higher organisms by the oxidation of one of the terminal guanidino nitrogen atoms of L-arginine. This process is catalysed by the enzyme NOS. Depending on the microenvironment, NO can be converted to various other reactive nitrogen species (RNS) such as nitrosonium cation (NO⁺), nitroxyl anion (NO⁻) or peroxynitrite (ONOO⁻).
Oxygen free radical (OFR):

These are not the only important free radicals in biochemistry, although they are often the initial species formed. Other free radicals of importance are wide range of carbon centered radicals that arise from they attach of an oxidizing radical on a biomolecule such as lipid, nucleic acid, proteins, carbohydrates. These react with oxygen very rapidly to form the corresponding peroxo radicals. Sulphur containing free radicals such as “Thiol radicals” formed during the oxidation of glutathione^{100}.

Free Radical Induced Cell Injury:

ODFR (oxygen derived free radicals) are highly reactive species with, OH as one of the most reactive free radicals. In-vitro and In-vivo studies have shown that ODFR can produce chemical modification proteins, lipids, carbohydrates and nucleotides with varying cytotoxic effects^{101}.

Addition of electrons to molecular oxygen leads to the genesis to a series of reactive molecules collectively called reactive oxygen intermediates (ROI). These are capable of injuring tissue and be responsible for necrosis. The most significant ROI are superoxide anion (O_{2}^{-}), perhydroxyl radical (HO_{2}^{+•}), the peroxide ion (O_{2}), hydroxyl radical (OH^{•}) and the hydroxyl anion (OH^{-}).
ROI react with themselves and other molecules available within tissues to make further molecular species. These are capable of inflicting cell injury in their own right, for example,

\[ \text{O}_2^{+} + \text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O}^{+} + \text{O}_2 \]

This is Haber-Weiss reaction, and is accelerated by metal ions such as Fe\(^{2+}\) in metalloproteins.

\[ 2\text{O}_2^{••} + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2^{+} + \text{O}_2 \]

This is dismutation reaction, and is accelerated by enzymed superoxide dismutase. As we have discussed earlier that ROI may cause cell injury and responsible for cell necrosis, in can be depicted from the figure below.
Figure 15: Generation of ROI and its Effects

Cell Injury at Molecular Level:

Among all major class of biomolecules like protein, lipids, carbohydrates, nucleotides etc, lipids are most susceptible towards free radical attack and this leads to lipid peroxidation.

Lipid Peroxidation:

Free radical specially acts on polyunsaturated fatty acids (PUFA), either directly or through the formation of reactive oxygen species.
Figure 16: Formation and Propagation of Lipid Radicals Leading to Lipid Peroxidation

Peroxidation of membrane lipid results in the formation lipid peroxy radical (ROO\(^-\)) which then produce lipid hydroperoxide (ROOH). The peroxy radical is unstable and readily undergoes decomposition, catalyzed by transition metals ions, particularly Fe\(^{3+}\), to form additional radical products. Further, lipid peroxy radical (ROO\(^-\)), lipid radicals (R\(^-\)), lipid peroxidation may be mediated by OH\(^-\), or though the formation of an ADP-perferferly ion complex (ADP-Fe\(^{2+}\)-O\(_2\)), ADP-Fe\(^{3+}\)-O\(_2\)). Superoxide (O\(_2^{-}\)) may also initiate lipid peroxidation through the intermediate formation of singlet oxygen. The breakdown of lipid hydroperoxidases (ROOH) may also liberate singlet oxygen, which will react with other unsaturated lipids to form additional lipid hydroperoxides.
**Protein Oxidation:** Thiol containing proteins are particularly susceptible to peroxidation damage. This may have special relevance in the genesis of disturbed cellular in homeostasis, since the Ca-ATPase and Na-ATPase of plasma membranes are both thiol containing proteins. Further, binding of transition metal ions at specific site in protein results in specific damage. This type of specific metal binding is called as “site specific damage”.

Moreover, proteins and nucleic acid appear less susceptible than PUFA to free radical attack. Random attack of radicals on proteins is unlikely to very damaging unless very extensive.

**Carbohydrate Oxidation:** Free radical induced oxidation of carbohydrate result in the formation of oxaldehyde from monosaccharide sugar. These oxaldehyde have been implicated in proteins aggregation. ODFR may also depolymerize carbohydrate polymer such as hyaluronic acid, which is responsible for maintenance of high viscosity of synovial fluid and thus may have role in rheumatoid arthritis.

**DNA Oxidation:** ROI causes strand breaks and has the secondary effect of inducing the enzyme poy (ADP-ribose) polymerase. It has been suggested that the resulting depletion of cellular ADP may be sufficiently sever to reduce total cellular adenine nucleotide (including ATP) to critical levels.
Table No. 10 shows examples of injurious stimuli producing cell injury

**Table 10: Noxious stimuli producing cell injury**

<table>
<thead>
<tr>
<th>Noxious stimulus</th>
<th>Cell types affected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adrimycin</td>
<td>Cardiac</td>
</tr>
<tr>
<td>Ischemia/reperfusion</td>
<td>Neuronal/cardiac/endothelial</td>
</tr>
<tr>
<td>Mercury</td>
<td>Renal/neuronal</td>
</tr>
<tr>
<td>Menadione</td>
<td>Hepatic</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Hepatic</td>
</tr>
<tr>
<td>Carbon tetrachloride</td>
<td>Hepatic / renal</td>
</tr>
<tr>
<td>Cepaloridine</td>
<td>Renal</td>
</tr>
<tr>
<td>Alloxan</td>
<td>Pancreatic</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>Epithelial</td>
</tr>
</tbody>
</table>

**Medicinal Plants Used for the Treatment of Hepatoprotective Activity**

It is estimated that about 7,500 plants are used in local health traditions in, mostly, rural and tribal villages of India. Out of these, the real medicinal value of over 4,000 plants is either little known or hitherto unknown to the mainstream population. The classical systems of medicine such as Ayurveda, Siddha, Amchi, Unani and Tibetan use about 1,200 plants. A detailed investigation and documentation of plants used in local health traditions and pharmacological evaluation of these plants and their taxonomical relatives can lead to the development of invaluable plant drugs for many dreaded diseases.
Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The Indian Traditional Medicine like Ayurveda, Siddha and Unani are predominantly based on the use of plant materials. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases. One of the important and well documented uses of plant products is their use as hepatoprotective agents. Hence, there is an ever increasing need for safe hepatoprotective agent. In spite of tremendous strides in modern medicine, there are hardly any drugs that stimulate liver function, offer protection to the liver from damage or help regeneration of hepatic cell. Many formulations containing herbal extracts are sold in the Indian market for liver disorders.

A large number of plants and formulations have been claimed to have hepatoprotective activity. Nearly 160 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. In India, more than 87 plants are used in 33 patented and proprietary multi ingredient plant formulations. In spite of the tremendous advances made, no significant and safe hepatoprotective agents are available in modern therapeutics. Therefore, due importance has been given globally to develop plant-based hepatoprotective drugs effective against a variety of liver disorders.
**Table 11: Medicinal Plants Used for the Treatment of Hepatoprotective Activity**

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Family</th>
<th>Parts Used</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Achillea millefoliiven</em> Linn.</td>
<td>Compositae</td>
<td>Whole Plant</td>
</tr>
<tr>
<td><em>Aconitum herterophyllum</em> wall</td>
<td>Ranunculaceae</td>
<td>Root</td>
</tr>
<tr>
<td><em>Aegal marimelos</em> Corr.</td>
<td>Rutaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Aegiceras corniculatum</em></td>
<td>Aegicerataceae</td>
<td>Stem</td>
</tr>
<tr>
<td><em>Allium sativum</em> Linn.</td>
<td>Liliaceae</td>
<td>Bulb</td>
</tr>
<tr>
<td><em>Berberis lycium</em> Royle</td>
<td>Berberidaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Boerhaavia diffusa</em> Linn.</td>
<td>Nyctaginaceae</td>
<td>Root</td>
</tr>
<tr>
<td><em>Bryonia alba</em> Linn.</td>
<td>Cucurbitaceae</td>
<td>Root</td>
</tr>
<tr>
<td><em>Calotropis gigantea</em> (Linn)R.Br.</td>
<td>Asclepiadaceae</td>
<td>latex, flower, stem</td>
</tr>
<tr>
<td><em>Canavalia ensiformis</em> DC</td>
<td>Leguminosae</td>
<td>Root</td>
</tr>
<tr>
<td><em>Carapa Guianensis</em> Aublet</td>
<td>Meliaceae</td>
<td>Seed</td>
</tr>
<tr>
<td><em>Carthamus tinctorius</em> Linn.</td>
<td>Compositae</td>
<td>Flower</td>
</tr>
<tr>
<td><em>Cephaelis ipecacuanha</em> Rich.</td>
<td>Rubiaceae</td>
<td>Draught</td>
</tr>
<tr>
<td><em>Delphinium zalil</em> Atich &amp; Hemse</td>
<td>Ranunculaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td><em>Desmodium biflorum</em> Linn.</td>
<td>Fabaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td><em>Eclipta alba</em> Hassk.</td>
<td>Compositeae</td>
<td>Plant juice</td>
</tr>
<tr>
<td><em>Emblica officinalis</em> Gaertn</td>
<td>Euphorbiaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Euphorbia neriifolia</em> Linn.</td>
<td>Euphorbiaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Ferula alliaceae</em> boiss.</td>
<td>Umbelliferae</td>
<td>Gum resin</td>
</tr>
<tr>
<td><em>Ficus asperrima</em> Roxb</td>
<td>Moraceae</td>
<td>Juice and bark</td>
</tr>
<tr>
<td>Species</td>
<td>Family</td>
<td>Part</td>
</tr>
<tr>
<td>----------------------------------------------</td>
<td>-----------------</td>
<td>------------</td>
</tr>
<tr>
<td><em>Ficus benjamina</em> Linn</td>
<td>Moraceae</td>
<td>Bark juice</td>
</tr>
<tr>
<td><em>Ficus carica</em> Linn.</td>
<td>Moraceae</td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Ficus heterophylla</em> Linn. F</td>
<td>Moraceae</td>
<td>Root juice</td>
</tr>
<tr>
<td><em>Garcinia indica</em> chois.</td>
<td>Guttiferae</td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Garcinia kola</em> Heckel</td>
<td>Guttiferae</td>
<td>Seeds</td>
</tr>
<tr>
<td><em>Gentiana kurroo</em> Royld</td>
<td>Gentianaceae</td>
<td>Root</td>
</tr>
<tr>
<td><em>Hedyotis corymbosa</em> Linn.</td>
<td>Asclepiadaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Hemidesmus indicus</em></td>
<td>Rubiaceae</td>
<td>Whole plant</td>
</tr>
<tr>
<td><em>Lawsonia inermis</em> Linn</td>
<td>Lythraceae</td>
<td>Bark</td>
</tr>
<tr>
<td><em>Luffa echinata</em> Roxb.</td>
<td>Cucurbitaceae</td>
<td>Fruit and seed</td>
</tr>
<tr>
<td><em>Lycopersicon esculentum</em> Mill.</td>
<td>Solanceae</td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Mentha longifolia</em> Linn</td>
<td>Labiatae</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Momordica cochimchinesis</em> spreng</td>
<td>Cucurbitaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td><em>Moringa oleifera</em> Lam.</td>
<td>Moringaceae</td>
<td>Root</td>
</tr>
<tr>
<td><em>Murraya koenigii</em> Linn</td>
<td>Rutaceae</td>
<td>Leaves</td>
</tr>
<tr>
<td><em>Nelumbo mucifera</em> Gaertn.</td>
<td>Nymphaceae</td>
<td>Flower</td>
</tr>
<tr>
<td><em>Paeonia emodi</em> Wall</td>
<td>Ranunculaceae</td>
<td>Tubers</td>
</tr>
<tr>
<td><em>Phyllanthus niruri</em> Linn.</td>
<td>Euphorbiaceae</td>
<td>Plant</td>
</tr>
<tr>
<td><em>Picrorhiza kurroa</em> Royle.</td>
<td>Scrophulariaceae</td>
<td>Root</td>
</tr>
<tr>
<td><em>Rhem emodi</em> Wall.</td>
<td>Polygonaceae</td>
<td>Rhizome</td>
</tr>
<tr>
<td><em>Rumex crispus</em> Linn.</td>
<td>Polygonaceae</td>
<td>Root</td>
</tr>
<tr>
<td><em>Solanum dulcamara</em> Linn.</td>
<td>Solanaceae</td>
<td>Berries</td>
</tr>
<tr>
<td><em>Solanum indicum</em> Linn.</td>
<td>Solanaceae</td>
<td>Fruit, plant</td>
</tr>
<tr>
<td><em>Solanum nigrum</em> Linn.</td>
<td>Solanaceae</td>
<td>Dried fruit</td>
</tr>
<tr>
<td><em>Taraxacum officinale</em> Weber.</td>
<td>Compositae</td>
<td>Root</td>
</tr>
<tr>
<td><em>Terminalia chebula</em> Retz</td>
<td>Combretaceae</td>
<td>Fruit</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Part</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------</td>
<td>------------</td>
</tr>
<tr>
<td><em>Tinospora cordifolia</em> Willd.</td>
<td>Menispermaceae</td>
<td>Stem</td>
</tr>
<tr>
<td><em>Vitex negundo</em> Linn.</td>
<td>Verbenaceae</td>
<td>Plant</td>
</tr>
<tr>
<td><em>Woodfordia fruticosa</em> Kurz.</td>
<td>Lythraceae</td>
<td>Flower</td>
</tr>
<tr>
<td><em>Zinziber officinale</em> Rose</td>
<td>Zingiberaceae</td>
<td>Rhizome</td>
</tr>
</tbody>
</table>