CHAPTER 2

Literature Survey

2.1 Literature Review of Leucas aspera (Wild.) Link.

2.1.1 Taxonomical Classification\textsuperscript{35, 36, 37}

Table 2.1: Scientific Classification of Leucas aspera

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae, Plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta, Vascular plant</td>
</tr>
<tr>
<td>Super division</td>
<td>Spermatophyta, Seed plant</td>
</tr>
<tr>
<td>Division</td>
<td>Angiosperma</td>
</tr>
<tr>
<td>Class</td>
<td>Dicotyledonae</td>
</tr>
<tr>
<td>Sub-class</td>
<td>Gamopetalae</td>
</tr>
<tr>
<td>Series</td>
<td>Bicarpellatae</td>
</tr>
<tr>
<td>Order</td>
<td>Tubiflorae</td>
</tr>
<tr>
<td>Family</td>
<td>Lamiaceae</td>
</tr>
<tr>
<td>Genus</td>
<td>Leucas</td>
</tr>
<tr>
<td>Species</td>
<td>Leucas aspera (Willd.) Link</td>
</tr>
</tbody>
</table>

Botanical Name: Leucas aspera (Willd.) Link

Synonym\textsuperscript{38}:

Leucas dimidiata (Roth)

Leucas dimidiata (Benth)

Leucas minahassae (Koord)
Leucas obliqua (Buch.-Ham)

Leucas plukenetii (Roth) Spreng.

Phlomis aspera (Willd)

Phlomis dimidiata (Roth)

Phlomis esculenta (Roxb)

Phlomis obliqua (Buch.-Ham)

Phlomis plukenetii (Roth)

**Family:** Lamiaceae

**Common Names**:

Sanskrit : Dronapushpi, Chitrapathrika, Chitrak-shupa

Punjabi : Guldora

Bengali : Darunaphula, Hulkasha

Gujarati : Kulnnphul

Hindi : Goma madhupati

Sindhi : Kubo

Maharashtra : Bahuphul

Bombay : Tumba

Telugu : Tunni

**Distribution**: Leucas aspera is originate in India (from the Himalayas down to Ceylon), Philippines, Africa, China, Malaysia, Nepal, Pakistan, Sri Lanka, Myanmar and Thailand.
2.1.2 Plant Description:

*Leucas aspera* is a yearly, branched, herb. The height is 15-60 cm with quadrangular stem and branches.

**Leaves**

The leaves of the *Leucas aspera* can be obtuse, linear or petiolate. They can reach up to lengths of 8.0 cm, and be 1.25 cm broad. The measurement lengthwise of petioles is typically 2.5 to 6 mm lengthy. The leaves epidermis is enclosed in a thick waxy cuticle and is traversed with stomata.\(^{36}\)

**Stem**

The epidermis of the stem is enclosed in a thick waxy cuticle and contains few traversed stomata. Normally in younger stems, the xylem tissue is radially structured and the parenchymatous pholem tissue is very thin. As the stem ages the pholem tissue widens and can be establishing on both sides of the radial xylem tissue.
Roots

The root of the *Leucas aspera* contains epidermal cells which are very thin and narrowly packed together. The cell walls of the epidermal cells are very lean, compressed and in a straight line. The parenchyma in the cortex contains broad walls. The parenchyma cells are polygonally shaped and contain a huge amount of starch grains. The cambium separates the phloem and xylem.

Flowers

Flowers on the *Leucas aspera* are white, small and in a straight line attached to the base without a peduncleor stalk. The flowers are held jointly in auxiliary whorls or dense terminals. They have 6 mm extended bracts that are bristle-tipped, linear and have long slender hairs.

Calyx

The length of calyx is 8 to 13 mm. It has a tubular shape. The bottom half is glabrous and membraneous, upper half is hispid and ribbed. It has a small mouth and is very oblique. It has little, short triangular teeth.

Corolla

The corolla of *Leucas aspera* is 1 cm in length and the tube is 5 mm in length. It is annulate in the middle portion and pubescent on the upper region. The calyx is "densely white-woolly", upper lip is just about 3 mm in length and the lower lip is just about 6 mm in length.
The middle lobe is curved and the sideways lobes are subacute and small in size.

**Fruit**

The fruit of the *Leucas aspera* is 2.5 mm long. They are nutlets type which is brown, smooth and oblong in shape. The outer portion of the fruit is curved while the inner portion is angular.\(^{36, 41}\)

**2.1.3 Traditional Uses:**

*Leucas aspera* is a used as stimulant, anthelmintic, laxative and diaphoretic. It is also used for the treatment of asthma and bronchitis. Hot water extract of whole plant is used for the treatment of inflammation and jaundice. Whole plant extract is used orally to treat scabies, and snake bite. *Leucas aspera* is externally used as an insect repellant. The flowers fried in ghee are given orally for management of cough and colds.

The aroma of flowers is inhaled in the contradictory nostril for the management of migraine. The juice of leaves is used for treatment of ear pain and for pus release from ear. The paste of leaves with chalk is applied to tooth cavity to avoid decay. The water extract of leaves is used as an antivenin. Externally infusion of leaves is used to cure scabies. Leaf paste with turmeric is used to heal wounds. The water extract of roots, stem of *Leucas aspera* is used orally for high fevers, and malarial fevers.\(^{38, 42-50}\)
2.1.4 Reported Phytochemical Constituents:

Srinivasan R. et al., (2011) have discovered presence of triterpenoids in whole plant. Entire plant is reported to have oleanolic acid, ursolic acid and 3-sitosterol. Prajapati M.S et al., (2010) have reported to have nicotine, sterols, two new alkaloids (compound α-sitosterol, m.p. 61-2° and β-sitosterol, m.p.183-4°), reducing sugars (galactose), glucoside (230-1°), diterpenes (leucasperones A and B, leucasperols A and B and isopimarane glycosides (leucasperosides A, B and C) in aerial parts. Aerial parts also contain other compounds like a sperphenamate, maslinic acid, (-)-isololiolide, linfolioside, nectandrin B, meso-dihydroguaiaretic acid, macelignan, acacetin, apigenin-7-O-[6'-O-(p-coumaroy-l)-3-D-glucoside], chrysoeriol, apigenin, erythro-2-(4-allyl-2,6-dimethoxyphenoxy)-1-(4-hydroxy-3-methoxyphenyl)propanol-1, myristargenol B. U-farnesene (26.4%), x-thujene (12.6%) and menthol (11.3%) were the main constituents.

Misra T.N. et al., (1992) have reported as the flower to have 10 compounds; among them amy-propionate (15.2%) and isoamy-l-propionate (14.4%) were central. Pradhan B. et al., (1990) have reported as seed contain palmitic acid (6.25%), stearic acid (2.84%), oleic acid (42.07%), linoleic acid (48.1%), and linolenic acid (0.65%). The unsaponifiable part have 3-sitosterol and ceryl alcohol.

Thakur D.K. et al., (1987) have reported as shoot contain novel phenolic compounds (4-(24-hydroxy-1-oxo-5-npropyltetracosany-l)-phenol), aliphatic ketols (28-hydroxypentatriacontan-7-one, 7-
hydroxydotriacontan-2-one), long-chain compounds (1-hydroxy-tetratriacontan-4-one, 32-methy-tetratriacontan-8-ol), nonatriacontane, 5-acetoxy triacontane, β-si tosterol and dotriacontanol.53

Xavier J. et al., (2013) have reported as Leucolactone (I) extracted from the root of Leucas aspera have been characterized as 3, 3, 16c-dihydroxy oleanan-28 -1, 3-olide.54

2.1.5 Reported biological activities:

Atchutkumar K. et al., (2013) studied antihyperglycemic activity of methanolic extract of Leucas aspera wild whole plant on blood glucose levels of streptozotocin-induced diabetic rats.55 Xavier J. et al., (2013) studied phytochemical, pharmacological and antiinsecticidal activity of Leucas aspera (wild.) linn.54


Sandeep Banu. et al., (2012) studied hepatoprotective and antioxidant activity of *Leucas aspera* against Dgalactosamine induced liver damage in rats.


2.2 Literature Review of *Cassia tora* L.

2.2.1 Taxonomical Classification

**Table 2.2: Scientific Classification of *Cassia tora* $^{74}$**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
</tr>
</thead>
<tbody>
<tr>
<td>Division</td>
<td>Magnoliphyta</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida</td>
</tr>
<tr>
<td>Sub-class</td>
<td>Rosidae</td>
</tr>
<tr>
<td>Order</td>
<td>Fabales</td>
</tr>
<tr>
<td>Family</td>
<td><em>Caesalpiniaceae</em></td>
</tr>
<tr>
<td>Genus</td>
<td><em>Cassia</em></td>
</tr>
<tr>
<td>Species</td>
<td><em>Cassia tora</em> L.</td>
</tr>
</tbody>
</table>

**Botanical Name:** *Cassia tora* L.

**Synonym$^{74}$:**

*Cassia borneensis* (Miq.)

*Cassia gallinaria* (Collad.)

*Cassia numilis* (Collad.)

*Emelista tora*

*Cassia tora var. borneensis* (Miq)

*Senna tora* (L) Roxb

**Family:** Leguminosae
**Common Names**:

- Hindi: Charota, Chakvad, Chakavat.
- Bengali & Oriya: Chakunda
- Gujarati: Kawaria
- Canarese: Gandutogache
- Malyalam: Chakramandrakam, takara
- Marathi: Takala
- Sanskrit: Chakramarda, Dadmari, Dadrughra, Taga
- Tamil: Tagarai
- Telugu: Chinnakasinda

**English Name**: Foetid cassia, The Sickle Senna, Wild Senna

**Distribution**:
*Cassia tora* is originated in India, Pakistan, China, Bangladesh, Sri Lanka and South America.

**Figure 2.2: Whole Plant and roots of *Cassia tora* L.**

**2.2.2 Plant Description**:

*Cassia* tora is a wild crop and grown in most parts of India as a weed. It is a yearly foetide herb, 30-90cm elevated. Leaves are pinnate,
up to 10cm long rachis grooved. There is conical gland between each of two lowest pairs of leaflet. Leaflets are in 3 pairs, opposite, abovate, oblong and base ablique. Flowers are in pair in axis of leaves. Petals are five having pale yellow colour. Fruit are pod types which are obliquely separate.

**2.2.3 Traditional Uses**\(^{76, 77}\)

Usually it is used as carminative and stimulant. Its leaves, seeds, and roots are used medicinally, mainly in Asia. As a folk remedy, the seeds are often roasted, then boiled in water to produce a tea. Roasted and ground, the seeds have also been used as a substitute for coffee. According to Ayurveda the leaves and seeds are acrid, antiperiodic, anthelmintic, liver tonic, cardiotonic and expectorant. The leaves and seeds are helpful in leprosy, ringworm, flatulence, colic, dyspepsia, constipation, cough, bronchitis, cardiac disorders.

*Cassia tora* powder made from *cassia tora* seeds and *cassia tora* splits are some ancient natural ingredients. In India, *Cassia tora* is used as a natural insect killer in organic farms. Roasted seeds are used as alternate for coffee, like *tephrosia* seeds. *Cassia tora* powder is most commonly used in the pet-food manufacturing. It is mix with guar gum for use in mining and other industrial application.

According to Chinese Material Medica, it promotes blood circulation, and its cold nature makes it effective in the management of heat syndromes.
2.2.4 Reported Phytochemical Constituents⁷⁶, ⁷⁹-⁸¹:

Smita Jain et al., (2010) have reported eight compounds which are isolated from the ethyl acetate fraction of 95% EtOH extract of the transformed roots of Cassia obtusifolia. They are betulinic acid, chrysophanol, stigmasterol, 1-hydroxy-7-methoxy-3-methyl-anthraquinone, 8-O-methylchrysophanol, 1-O-methylchrysophanol and aloe- emodin.

Pranjali Ranaware et al., (2012) have investigated thirteen phenolic glycoside including six new compounds which are isolated from seeds of Cassia tora (Leguminosae). These new compounds are rubrofusarin triglucoside, nor-rubrofusarin gentiobioside, demethyflavasperone-gentiobioside, torachrysone-gentiobioside, and torachrysone-tetraglucoside and torachrysone- apioglucoside. These compounds were elucidated on the basis of spectroscopic and chemical evidence.

Yogesh Shakywar et al., (2011) have investigated two new naphtha-pyrone glycosides which are 9(beta-D-glucopyranosyl-(1—6)-O-beta-Dglucopyranosyl)oxy]-10-hydroxy-7-methoxy-3-mehtyl-1H-napthopyran-1-one and 6-O-beta-D-glucopyranosyl)oxy]-rubrofusarin.

Meena A.K. et al., (2010), emodin, tricontan-1-ol, stigmasterol, β-sistosteral-β-D glucoside, freindlen, palmitic, stearic, succinic and d-tartaric acids uridine, quercitrin and isoquercitrin are isolated from leaves.
2.2.5 Reported biological activities:

Manmohan Singhal et al., (2012) have investigated Cassia tora L. Creams inhibit Psoriasis in Mouse Tail Model. Yogesh Shakywar et al., (2011) have investigated pharmacognostical properties and their traditional uses of Cassia tora Linn. They have also investigated antioxidant, hepatoprotective, antigenotoxic, anthelmintic, antifungal activities.


2.3 Antimicrobial activity:

2.3.1 Antimicrobial agents

An antimicrobial is substances that kills microorganisms or stops their growth. The grouping of antimicrobial agents can be done according to the type of microorganisms it acts on. For bacteria, antibacterials are used and for fungi, antifungals are used. Also on
the basis of their function, they can be classified into microbicidal and microbiostatic. Substances that kill microbes are called microbicidal, while those that merely inhibit their growth are called microbiostatic.

Disinfectants, antiseptics and antibiotics are the main classes of antimicrobial agents. Disinfectants kill a wide range of microbes on non-living surfaces to prevent the spread of illness. Antiseptics are applied to living tissue and help reduce infection during surgery and antibiotics destroy microorganisms within the body.

Plants have wonderful ability to synthesize aromatic substances; examples are phenols or their oxygen-substituted derivatives. In many cases, these substances have plant defense mechanisms against predation by microorganisms, insects, and herbivores.

2.3.2 Flavones, flavonoids, and flavonols.

Flavones are phenolic structures containing one carbonyl group (as opposed to the two carbonyls in quinones). A flavonol yields by addition of a 3-hydroxyl group. Flavonoids are also hydroxylated phenolic substances but arise as a C₆-C₃ unit linked to an aromatic ring. Since they are recognized to be synthesized by plants in response to microbial infection, it should not be amazing that they have been found in vitro to be effective antimicrobial substances against a wide range of microorganisms. Their activity is most likely due to their ability to complex with extracellular and soluble proteins and to
complex with bacterial cell walls. More lipophilic flavonoids may also disturb microbial membranes.

Flavonoid compounds demonstrate inhibitory effects against multiple viruses. Several studies have reported the effectiveness of flavonoids such as glycyrrhizin (from licorice) and chrysin against HIV. Flavonoids having no hydroxyl groups on their β-rings are more active against microorganisms than are those flavonoids having the −OH groups. This result confirms the idea that the membrane is the microbial target. Lipophilic compounds would be more disrupting of this structure. However, many authors have also found that the more hydroxylation, the superior the antimicrobial activity. It is safe to say that there is no clear certainty for the degree of hydroxylation and toxicity to microorganisms.

### 2.3.3 The microbiological assay

The term microbiological assay is a biological assay carried out with micro-organisms like yeast, bacteria, moulds, etc. In microbiological assay the relative potency or activity of compounds is calculated by determining the amount of test material required for producing stipulated effect on suitable organism under standard conditions.

The procedures employed in microbial assay were,

- Cylinder plate method or cup plate method
- Turbidimetric or tube assay method
Table 2.3: List of plants having the antimicrobial activity

<table>
<thead>
<tr>
<th>Botanical Name</th>
<th>Compound</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pimenta dioica</em></td>
<td>Essential oil</td>
<td>General</td>
</tr>
</tbody>
</table>
| *Aloe barbadensis,*  | Latex         | *Corynebacterium,*  
| *Aloe vera*        |               | *Salmonella,*  
|                 |               | *Streptococcus,*  
|                 |               | *S. aureus*                                                               |
| *Malus sylvestris* | Flavonoid     | General                                                                   |
| *Withania somniferum* | Lactone    | Bacteria, fungi                                                           |
| *Aegle marmelos*   | Terpenoid     | Fungi                                                                      |
| *Berberis vulgaris* | Alkaloid      | Bacteria, protozoa                                                        |
| *Ocimum basilicum* | Terpenoids    | *Salmonella,* bacteria                                                     |
| *Piper betel*      | Essential oils| General                                                                   |
| *Piper nigrum*     | Alkaloid      | Fungi, *Lactobacillus,*  
|                 |               | *Micrococcus,* *E. coli,*  
|                 |               | *E. faecalis*                                                             |
| *Barosma setulina* | Terpenoid     | General                                                                   |
| *Rhamnus purshiana* | Tannin       | Viruses, bacteria, fungi                                                  |
| *Capsicum annuum*  | Terpenoid     | General                                                                   |
| *Erythroxylum coca* | Alkaloid      | Gram-negative and -positive cocci                                         |
| *Allium sativum*   | Sulfoxide     | General                                                                   |
| *Panax notoginseng* | Saponins     | *E. coli,* *Sporothrix schenckii,*  
|                 |               | *Staphylococcus,* *Trichophyton*                                          |
| *Citrus paradisa*  | Terpenoid     | Fungi                                                                      |
2.4 Antioxidant Activity

2.4.1 Free radicals

Antioxidants are the substances which guard our cells against the effects of free radicals. Free radicals are Reactive oxygen group. These molecules are extremely reactive, which is having oxygen molecules. Hydroxyl radical, the super oxide anion radical, hydrogen peroxide, singlet oxygen, nitric oxide radical, hypochlorite radical, and different lipid peroxides are the different classes of Reactive oxygen species. These molecules have ability of reacting with membrane lipids, nucleic acids, proteins and enzymes which causes cellular damage.

In existing organisms different Reactive oxygen species are produced in various ways; for an example regular aerobic respiration, motivated polymorphonuclear leukocytes and peroxisomes. These are main endogenous income of the majority of the oxidants formed by cells. Exogenous media of free radicals are smoking, ionizing type of radiation, pollutants, organic solvents and different types of pesticides. Free radicals are defined as chemical genus allied with an odd or unpaired electron.

Free radicals are neutral, little lived, unstable and extremely reactive to pair up the odd electron and ultimately attain stable configuration. Free radicals have ability of offensive the healthy cells of the body, which results in to loose their structure and role. Aging, cancer, cardiovascular disease, immune system refuse, liver diseases,
diabetes mellitus, inflammation, renal failure, brain dysfunction and stress are the types of cell damage created by free radicals. Humans have an extremely special and complex antioxidant protection system, which neutralize free radicals. Thus, antioxidants have ability of deactivating, free radicals previous to they attack cells. Antioxidants are finally essential for maintaining finest cellular and complete health.

There is an active equilibrium between the quantity of free radicals formed in the body and antioxidants to scavenge them to guard the body against injurious effects. Under regular physiological circumstances, the quantity of antioxidant present is not sufficient to deactivate free radicals created. So, it is clear to improve food with antioxidants to protect against dangerous diseases. Therefore there has been an increased awareness in the food industry and in defensive medicine in the improvement of natural antioxidants.

2.4.2 Natural antioxidant

Natural antioxidants are normally obtained from plant materials. Hence plants having antioxidant properties are becoming more popular all over the world.

Table 2.4: List of plants having the antioxidant activity

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Plant Part</th>
<th>Chemical Constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allium sativum</td>
<td>Leaves, Buds</td>
<td>Gingerol, Ascorbis acid, Ginkgogolide</td>
</tr>
<tr>
<td>Zingiber officinalis</td>
<td>Leaves, Rhizomes</td>
<td>Alanine, carotene,</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Part(s)</td>
<td>Constituents</td>
</tr>
<tr>
<td>---------------------</td>
<td>------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td><em>Ginkgo biloba</em></td>
<td>Plant</td>
<td>Histidine, menthionine, Palmitic acid, Alanine, sitosterol</td>
</tr>
<tr>
<td><em>Vitis vinifera</em></td>
<td>Seed, fruit</td>
<td>Alanine, Histidine, menthionine, sitosterol</td>
</tr>
<tr>
<td><em>Piper nigrum</em></td>
<td>Fruit</td>
<td>Ascorbic acid carotene, Lauric acid, Piperine</td>
</tr>
<tr>
<td><em>Salvia sclarea</em></td>
<td>entire plant, seed</td>
<td>Palmitic acid, rosemarinic acid</td>
</tr>
<tr>
<td><em>Withania somnifera</em></td>
<td>Leaves, roots</td>
<td>Ascorbic acid, tocopherol</td>
</tr>
<tr>
<td><em>Citrus aurantifolia</em></td>
<td>Fruit</td>
<td>Alanine, sitosterol</td>
</tr>
<tr>
<td><em>Catharanthus roseus</em></td>
<td>Leaf</td>
<td>Tocopherol, kaempferol</td>
</tr>
<tr>
<td><em>Artemisia vulgaris</em></td>
<td>Flowering aerial part</td>
<td>Flavonol glycosides</td>
</tr>
<tr>
<td><em>Bellis perennis</em></td>
<td>Flowering aerial part</td>
<td>Flavonoids</td>
</tr>
<tr>
<td><em>Carum carvi</em></td>
<td>Roots, Flowering aerial part</td>
<td>Flavonoids</td>
</tr>
<tr>
<td><em>Calluna vulgaris</em></td>
<td>Flowering aerial part</td>
<td>Flavonoids</td>
</tr>
<tr>
<td><em>Vaccinium myrtillus</em></td>
<td>Flowering aerial part</td>
<td>Tannins, Flavonoids</td>
</tr>
<tr>
<td><em>Lotus corniculatus</em></td>
<td>Flowering aerial part</td>
<td>Flavonoids, Phenolic acid</td>
</tr>
<tr>
<td><em>Ononis spinosa</em></td>
<td>Flowering aerial part</td>
<td>Flavonoids</td>
</tr>
</tbody>
</table>
2.4.3 In vitro models for evaluating antioxidant activity

2.4.3.1) Conjugated diene assay

In this method conjugated dienes are quantitatively measured by measuring UV absorbance at 234 nm due to initial Polyunsaturated fatty acid (PUFA) oxidation. The theory of this assay is that, at the time of linoleic acid oxidation, the double bonds are changed into conjugated double bonds, which are measured by a strong UV absorption at 234 nm. The activity is recorded in expressions of Inhibitory concentration (IC$_{50}$).

2.4.3.2) 1, 1 diphenyl 2, picryl hydrazyl Method (DPPH)

This is the most widely used method for evaluation of antioxidant activity of numerous natural drugs. The principle of this method is that the decline of methanolic solution of colored free radical DPPH. The method includes measurement of decrease in absorbance of DPPH at its absorption maxima of 516 nm, which is proportional to concentration of free radical scavenger added to DPPH reagent solution. The activity is recorded as effective concentration (EC$_{50}$).

2.4.3.3) Super oxide radical scavenging activity

The principle of In-vitro super oxide radical scavenging activity is Nitro blue tetrazolium (NBT) reduction. Reduction of NBT is the mostly used procedure. The production of super oxide radical by auto-oxidation of riboflavin in presence of light is the principle of this
method. The super oxide radical decreases NBT to a blue colored formazan that can be measured at 560nm. The ability of extracts to restrain the colour to 50% is calculated in terms of EC$_{50}$.

2.4.3.4) Hydroxyl radical scavenging activity

The base of this method is *in-vitro* creation of hydroxyl radicals using Fe$^{3+}$/ascorbate/EDTA/H$_2$O$_2$ system. Scavenging of this hydroxyl radical in existence of antioxidant is calculated. The hydroxyl radicals produced by the oxidation is allow to react with dimethyl sulphoxide (DMSO) to give formaldehyde. Formaldehyde produced has intense yellow color after reating with Nash reagent (2M ammonium acetate with 0.05M acetic acid and 0.02M acetyl acetone in distilled water). The intensity of yellow color created is calculated at 412nm spectrophotometrically against reagent blank. The activity is recorded as percentage of hydroxyl radical scavenging.

2.4.3.5) Nitric oxide radical inhibition activity

Nitric oxide have unpaired electron. So it is a type of free radical and demonstrates significant reactivity’s with definite types of proteins and additional free radicals. The principle of this method is the inhibition of nitric oxide radical produced from sodium nitroprusside in buffer saline. The absorbance of the chromophore is measured at 546nm. The activity is recorded as percentage of decrease of nitric oxide.
2.4.3.6) Reducing Power Method

The base of reducing power method is to increase in the absorbance of the reaction mixture. Enhancement in the absorbance indicates rise in the antioxidant activity. In this method antioxidant compound produce a colored complex with potassium ferricyanide, trichloro acetic acid and ferric chloride. This colored complex is measured at 700nm. Rise in absorbance of the reaction mixture ensure the reducing power of the samples.

2.4.3.7) Phospho molybdenum Method

This is a quantitative method by the creation of phospho-molybdenum complex to determine antioxidant ability. The method is based on the decrease of Mo (VI) to Mo (V) by the sample analyte and subsequent creation of a green phosphate Mo (V) complex at acidic pH.

2.4.3.8) Peroxynitrite radical scavenging activity

A scavenging of peroxy radical has been created to calculate antioxidant activity. The scavenging activity is measured by observing the oxidation of dihydrorhodamine on a microplate fluorescence spectrophotometer at 485nm.

2.4.3.9) 2, 2-azinobis (3-ethyl benzothiazoline-6-sulfonicacid (ABTS) diamonium salt Method

It also allows the measurement of antioxidant activity of mixtures of substances and so helps to decide additive and synergistic
effects. The principle of this method is the dealings between antioxidant and ABTS+ radical cation which has a typical color viewing maxima at 645, 734 and 815nm.

2.4.3.10) N, N-dimethyl-p-phenylene diamine dihydrochloride (DMPD) Method

The base of this method is the decrease of buffered solution of colored DMPD in acetate buffer and ferric chloride. The procedure includes measurement of reduction in absorbance of DMPD in existence of scavengers at its absorption maxima of 505nm. The activity was recored as % decrease of DMPD.

2.4.3.11) Oxygen Radical Absorbance Capacity (ORAC)

This method determines the ability of a product or chemical to guard against potentially harmful free radicals. This analytical procedure calculates the capacity of a food, vitamin and nutritional supplement to defend against the attack by free radicals. In this test Trolox (a water-soluble analog of Vitamin E) is used as a standard for the determination of the Trolox Equivalent (TE). The ORAC value is then determined from the Trolox Equivalent and recored as ORAC units or value. If the ORAC value is elevated, the "Antioxidant Power" will be the superior. The principle of this assay is to creation of free radical with 2, 2-azobis 2-amido propane dihydrochloride (AAPH) and measurement of reduction in fluorescence in existence of free radical scavengers. After addition of AAPH to the test solution, the
fluorescence is recorded and the antioxidant activity is recorded as trolox equivalent.

2.4.3.12) b-Carotene Linoleate model

This is the fast methods used for evaluating antioxidants. The principle of this method is that Linoleic acid oxidized by Reactive Oxygen Species” (ROS) formed by oxygenated water. The products produced will start the β-carotene oxidation, which will cause discoloration. Antioxidants decrease the degree of discoloration, which is recorded at 434nm.

2.4.3.13) Xanthine oxidase method

This is the existing procedure used for screening of antioxidant activity. The percentage inhibition in the xanthine oxidase activity in attendance of antioxidants is recorded. Xanthine oxidase enzyme produces uric acid with super oxide radicals. The quantity of uric acid is recorded at 292nm.

2.4.3.14) Ferric Reducing Ability of Plasma (FRAP) Method

The antioxidative activity is calculated by recording the rise in absorbance due to the production of ferrous ions from FRAP reagent containing 2, 4, 6 – tri (2 – pyridyl) – s – triazine (TPTZ) and FeCl₃6H₂O. The absorbance is recorded spectrophotometrically at 595nm.
2.4.3.15) **Total radical trapping antioxidant parameter (TRAP)**

**Method**

2, 2'-azo–bis (2-amidino-propane) hydrochloride) (ABAP) is act as a radical producer. The quenching reaction is measured in presence of antioxidants. The antioxidative ability is determined by recording the setback in decoloration.

2.4.3.16) **Cytochrome C test**

Superoxide anions were determined by spectrophotometric method. Xanthine oxidase converts xanthine to uric acid and gives superoxide anions. These radicals openly decrease ferri-cytochrome C to ferro-cytochrome C, having an absorbance alter at 550nm. When test compounds showed antioxidant activity, there was a decline in the decrease of ferri-cytochrome C.

2.4.3.17) **Erythrocyte ghost system**

The principle of this method is the separation of erythrocytes ghost cells, the induction of lipid peroxidation with the help of erythrocyte ghosts and the induction of tetra-butyl hydroxy peroxide (t-BHP). Thio barbituric acid reactive substance (TBARS) created during the reaction is recorded at 535nm.
2.4.3.18) Microsomal lipid peroxidation or Thiobarbituric acid (TBA) assay

TBA test is regularly used for determination of the peroxidation of lipids. TBA reacts with MDA to create a pink chromagen, which can be detected by spectrophotometric method at 532nm.

2.5 Antiinflammatory Activity: 106, 107, 108

2.5.1 Inflammation

Inflammation is defined as the local reaction of living mammalian tissues to tissue injury caused by any agent. It is a body protection reaction in order to eliminate or limit the spread of harmful agent as well as to take away the consequent necroosed cells and tissues.

2.5.2 Signs of Inflammation

Redness, swelling, pain, loss of function are signs of inflammation.

2.5.3 Types of Inflammation

There are two types of inflammation

1. Acute inflammation
2. Chronic inflammation

2.5.3.1 Acute Inflammation

Acute inflammation is of small period and represents the premature body reaction. The changes in acute inflammation can be properly explained under the following two headings:

I. Vascular events
II. Cellular events

I. Vascular events

Modification in the microvasculature is the first reply to tissue injury. The alterations are haemodynamic changes and vascular permeability alteration.

a. Haemodynamic changes

The early characters of inflammatory response result from changes in the vascular flow and caliber of small blood vessels in injured tissue. The features of haemodynamic changes in inflammation are finest confirmed by the Lewis experiment. Lewis induced the changes in the skin of inner characteristic of forearm by firm stroking with a blunt point. The reaction so elicited is renowned as triple response or red line response including of the following:

i) Red line appears within a few seconds following stroking and results from local vasodilatation of capillaries and venules.

ii) Flare is the bright reddish appearance or flush adjoining the redline and outcome from vasodilatation of the adjacent arterioles.

iii) Wheal is the swelling or edema of the surrounding skin occurring due to transudation of fluid into the extravascular space. These features, thus, elicit the typical signs of inflammation, redness, heat, swelling and pain.

b. Vascular permeability

An edema fluid is gathered in and around the inflamed tissue in the interstitial compartment which comes from blood plasma by its
getaway through the endothelial wall of peripheral vascular bed. In the early stage, the escape of fluid is due to vasodilatation and consequent elevation in hydrostatic pressure. This is transudate in nature. But then, the typical inflammatory edema, exudates, appears by inflamed vascular permeability of microcirculation.

II. Cellular events

The cellular phase of inflammation consists of 2 processes:

a) Exudation of leucocytes

b) Phagocytosis

a. Exudation of leucocytes

The escape of leucocytes from the lumen of microvasculature to the interstitial tissue is the most important characteristic of inflammatory response. In acute inflammation, polymorphonuclear neutrophils (PMNs) include the first line of body defense, afterward by monocytes and macrophages.

b. Phagocytosis

Phagocytosis is defined as the process of engulfment of solid particulate material by the cells (cell-eating). The cells performing this function are called phagocytes. There are 2 main types of phagocytic cells:

i) Polymorphonuclear neutrophils (PMNs) which emerge early in acute inflammatory response also called as microphages.

ii) Circulating monocytes and fixed tissue mononuclear phagocytes called as macrophages.
2.5.3.2 Chronic Inflammation

Chronic inflammation is defined as an extended process in which tissue destruction and inflammation occur at the same time.

a) Causes of chronic inflammation

1. Chronic inflammation following acute inflammation: when the tissue destruction is extensive or the bacteria survive and continue in small numbers at the site of acute inflammation e.g.: in osteomyelitis, pneumonia terminating in lung abscess.

2. Recurrent attacks of acute inflammation: when frequent bouts of acute inflammation culminate in chronicity of the process leading to chronic inflammation e.g. in recurrent urinary tract infection leading to chronic pyelonephritis, repeated acute infection of gall bladder.

3. Chronic inflammation starting de novo: when the infection with organism of low pathogenicity is chronic from the start e.g. infection with *mycobacterium tuberculosis*.

b) General features of chronic inflammation

Even if there may be differences in chronic inflammatory response depending upon the tissue concerned and causative organisms, there are some fundamental similarities amongst different types of chronic inflammation. These common features characterize any chronic inflammation.

1. Mononuclear cell infiltration: Chronic inflammatory lesions are infiltrated by mononuclear inflammatory cells like phagocytes and lymphoid cells. Phagocytes are represented by circulating monocytes, tissue macrophages, epithelioid cells and sometimes, multinucleated
giant cells. The macrophages encompass the most important cells in chronic inflammation.

2. **Tissue destruction or necrosis:** Tissue destruction and necrosis are general in many chronic inflammatory lesions and are brought about by activated macrophages by release of an array of biologically active substances.

3. **Proliferative changes:** As a result of necrosis, proliferation of small blood vessels and fibroblasts is encouraged resulting in development of inflammatory granulation tissue. Ultimately, healing by fibrosis and collagen lying takes place.

c) **Types of chronic inflammation**

1. **Nonspecific:** Nonspecific type of chronic inflammation occur when the irritant substances produces a non-specific chronic inflammatory reaction with formation of granulation tissue and healing by fibrosis e.g. chronic osteomyelitis, chronic ulcer.

2. **Specific:** Specific type of chronic inflammation occur when the harmful agent causes a typical histologic tissue response e.g. tuberculosis, leprosy, syphilis. However, for a more significant classification, histological characteristics are used for classifying chronic inflammation into 2 corresponding types;

   a. **Chronic nonspecific inflammation:** It is characterized by nonspecific inflammatory cell infiltration e.g. chronic osteomyelitis, lung abscess.

   b. **Chronic granulomatous inflammation:** It is characterized by formation of granulomas e.g. tuberculosis, leprosy, syphilis etc.
2.5.4 Chemical mediators of inflammation

I. Cell derived mediators

1. Vasoactive amines (Histamine, 5-HT)

2. Arachidonic acid metabolites
   i. Metabolites via cyclo-oxygenase pathway (prostaglandins, thromboxane A2, prostacyclin)
   ii. Metabolites via lipo-oxygenase pathway (5-HETE, leukotrienes)

3. Lysosomal components

4. Platelet activating factor

5. Cytokines (IL-1, TNF-α, TNF-β, IF-γ, chemokines)

6. Nitric oxide and oxygen metabolites

II. Plasma derived mediators (plasma proteases)

These are products of:

1. The kinin system

2. The clotting system

3. The fibrinolytic system

4. The complement system

2.5.5 The inflammatory cells

The cells involved in acute and chronic inflammation are circulating leucocytes, plasma cells and tissue macrophages.

a. Polymorphonuclear Neutrophils (PMN)

   Neutrophils or polymorphs along with basophils are identified as granulocytes due to the occurrence of granules in the cytoplasm. These granules have many proteases, myeloperoxidase, lysozyme, esterase, aryl sulfatase, alkaline phosphatase and cationic proteins.
The span of neutrophils ranges from 10-15μm and are dynamically motile. These cells include 40-75% of circulating leucocytes and their number is enlarged in blood and tissues in acute bacterial infections. These cells occur in the one marrow from stem cells. The functions of neutrophils in inflammation are as follows:

i) Preliminary phagocytosis of micro-organism as they form the primary line of body defense in bacterial infection.

ii) Engulfment of antigen-antibody complexes and non-microbial substance.

iii) Injurious effect of neutrophils is destruction of the basement membranes of glomeruli and small blood vessels.

b. Eosinophils

These are bigger than neutrophils but are less in number, comprising 1-6% of total blood leucocytes. Eosinophils share many structural and functional similarities with neutrophils like their creation in the bone marrow, locomotion, phagocytosis, lobed nucleus and existence of granules in the cytoplasm containing an array of enzymes of which main fundamental protein and eosinophil cationic protein are the most significant which have bactericidal and toxic action against helminthic parasites.

c. Basophils

The basophils include about 1% of circulating leucocytes and are morphologically and pharmacologically like to mast cells of tissue. These cells include coarse basophilic granules in the cytoplasm and a
polymorphonuclear nucleus. The roles of these cells in inflammation are:

i) In immediate and delayed type hypersensitivity reactions

ii) Release of histamine by IgE-sensitised basophils

d. Lymphocytes

Next to neutrophils, these cells are most abundant of the circulating leucocytes (20-45%). Apart from blood, lymphocytes are present in huge numbers in spleen, thymus, lymph nodes and mucosa-associated lymphoid tissue (MALT). They have scanty cytoplasm and consist almost exclusively of nucleus. Besides their task in antibody formation and in cell-mediated immunity these cells involve in the following types of inflammatory responses;

i) In tissues, they are dominant cells in chronic inflammation and late stage of acute inflammation.

ii) In blood, their number is increased (lymphocytosis) in chronic infections like tuberculosis.

e. Plasma cells

These cells are bigger than lymphocytes with richer cytoplasm and a nucleus which has cart-wheel pattern of chromatin. Plasma cells are not seen in peripheral blood. They build up from lymphocytes and are wealthy in RNA and γ- globulin in their cytoplasm.

f. Mononuclear-phagocyte system (Reticuloendothelial system)

This cell system includes cells derived from two sources with common morphology, function and origin. These are as under:
i) **Blood monocytes**: Theses comprise 4-8% of circulating leucocytes.

ii) **Tissue macrophages**

Role of macrophages in inflammation

i) Phagocytosis (cell eating) and Pinocytosis (cell drinking)

ii) Macrophages on activation by lymphokines released by T lymphocytes or by non-immunologic stimuli elaborate an array of biologically active substances like proteases, plasminogen activators, products of complement, some coagulation factor, chemotactic agent etc.

**g. Giant cells**

When the macrophages are unsuccessful to deal with particles to be removed, they fuse jointly and form multinucleated giant cells. Various types of giant cells are seen in inflammation and in certain tumors. These are foreign body giant cells, Langhans’ giant cells, Touton giant cells, Tumor giant cells etc.

2.5.6 **Animal Models to Screen Anti-Inflammatory Activity**

*In vitro methods*\(^{109-111}\)

1. \(^3\)H-Bradykinin receptor binding

2. Substance P and the tachykinin family

3. \(^3\)H- substance P receptor binding

4. Neurokinin receptor binding

5. Assay of polymorph nuclear leukocyte chemotaxis *in vitro*

6. Constitute and inducible cellular arachidonic acid metabolism *in vitro*

7. COX-1 and COX-2 inhibition
In vivo methods

1. Methods for testing acute and subacute inflammation
2. Ultraviolet erythema in guinea pigs
3. Vascular permeability
4. Rat paw edema
5. Pleuristy test
6. Granuloma pouch technique
7. Cotton wool granuloma
8. Measurement of gastric mucosal damage by intragastric inulin

2.6 Analgesic Activity

2.6.1 Pain

Pain is an unpleasant sensory and emotional experience associated with genuine and potential tissue damage. Sometimes, pain is difficult to exemplify. In reality, it can present in a different way for each person, which is why people identify and accept pain in different ways. The ability to deal with pain differs obviously from person to person. Some people can acknowledge severe pain without complaint, where others react strongly to it even though the pain may not be tremendous. This is why, in most cases, the reaction to pain is not always a reliable indicator of its real intensity. Unrelieved pain causes rise in cardiac work, metabolic rate, water retention, lowers oxygen levels, alter wound healing, changes immune function. In postoperative patients chronic pain can holdup the return of regular gastric and bowel function.

Pain can be classified upon following basis:-
• Location of the pain
• Duration of the pain
• Underlying causes of pain
• Intensity of the pain

2.6.1.1 Location of Pain

Pain is normally classified by body location. Two overlapping schemes relate the pain to the precise anatomy and/or body system thought to be concerned. The anatomic pain classification system recognises sites of pain as viewed from a regional viewpoint (eg, headache, pelvic pain). In dissimilarity, the body system pain classification method is based on classical body systems (eg, neurologic, vascular).120

2.6.1.2 Duration of Pain

The duration of the pain line is the clearest characteristic that can be made when classifying pain symptoms. Usually, acute pain is limited to pain of less than 30 days period, whereas chronic pain persists for more than 6 months. 121-122

2.6.1.3 Underlying cause of the pain123-124

1) Somatic Pain

Somatic pain is identified as musculoskeletal pain. It is inferred to be linked to ongoing activation of nociceptors that innervate somatic structures, such as joint, bone, muscle and connective tissue. It is preliminary in tissue such as skin and muscles as well as in joints, bones and ligaments. Somatic pain is normally characterized
as a pointed pain restricted in a specific area of injury. Swelling, cramping and bleeding may be with somatic pain. The pains that result from somatic processes are constant. This pain is acknowledged by recognition of a lesion and distinctiveness that typically include a well localized site and an experience described as aching, squeezing and stabbing.

II) Visceral Pain

Visceral pain is a diversity of nociceptive pain to be found inside the main body cavity due to injury or illness to an internal organ. Visceral pain is normally explained as generalized aching or squeezing. When injury is found, it sends signals to the spinal cord and brain. It is caused by compression in and around the organs, or by stretching of the abdominal cavity. The three most important centers for visceral pain are the thorax, abdomen and pelvis. The pain receptors in the visceral cavities react to stretching, swelling and oxygen deprivation. Visceral pain may radiate to further locations in the back and chest.

III) Neuropathic Pain

Neuropathic pain is usually caused by nerve damage such as that resulting from nerve compression or inflammation or from diabetes. Neuropathic pain is distinctive, for an example postherpetic neuralgia, and certain types of back and limb injuries. This type of pain is rigorous and usually explained as burning or tingling.
2.6.1.4 Intensity of Pain

A patient might rate the understanding of pain resulting from some pathologic condition as a 10, whereas another patient with the same pathology might explain the intensity of pain only as a 5, both using a 0 to 10 scale where 0 suggesting no pain at all and 10 representing the worse pain imaginable (Fig 2.3). But non–cancer related pain is frequently rated along a continuum (i.e., from mild, moderate, severe).

![Pain Rating Scale](image)

**Figure 2.3: Illustration of a Pain-rating Scale for Classifying the Intensity of Pain**

2.6.2 Mechanism of Pain:

2.6.2.1 Processing of Pain Signals.

As skin is damaged, nerve cell endings in the damaged area create a sensation of pain. These nerve cell endings are called nociceptors. Nociceptors are relatively unspecialized cell endings or “free endings” depending upon mylineation of these free fibers. They are additionally classified into type A-Fibers and type C fibers.

C-Fiber are non-myelinated fibers that conduct in the range of 0.5 m/s to 2 m/s and transmit noxious information from a variety of modalities including mechanical, thermal, and chemical stimuli. So they are termed as C-polymodal nociceptors.
A-Fibers are thinly myelinated fibers which conduct in the range of 2 m/s to 20 m/s. All fibers react to high intensity mechanical stimulation and are so termed high threshold mechanoreceptors. A few fibers also respond to thermal stimuli. So they are termed mechano–thermal receptors. A-fibers are supplementary subdivided into α, β, γ and δ fibers.

![Unmyelinated axon and Myelinated axon](image)

**Figure 2.4: Myelinated and Unmyelinated Fibers.**

A-delta group fibers are myelinated which allows them to conduct quicker action potentials. Their function explains why their speed is essential as they react mainly to sharp pressure or heat. The slower C fiber group of unmyelinated axons responds to strong stimulation to: pressure, heat or cold or noxious chemicals. This type of pain is the long-standing pain we experience after damage has already been done (Fig 2.5).¹²³
2.6.2.2 Pain pathways:

Exposure to an injurious stimulus activates nociceptors on the peripheral free nerve endings of most important afferent neurons. The cell bodies of these neurons bring together at the side of the spinal cord in the dorsal root ganglia and send one axon to the periphery and one to the dorsal horn of the spinal cord. With noxious stimulation substance-p, Glutamate, and other excitatory neurotransmitters are released.

Glutamate – This seems to be the foremost neurotransmitter when the threshold to pain is first crossed and is allied with acute pain.

Substance P – This is a peptide that contains 11 amino acids and is released by C fibers. It is normally allied with extreme, persistent,
chronic pain and used to pass on pain messages leading to a brain and the spinal. These neurotransmitters are released from the central terminations of the primary afferent fibers onto neurons of the spinal cord. Different terminals synapse directly on spinothalamic tract neurons in the dorsal horn which send long fibers up the contralateral side of the spinal cord to pass on pain impulses via ascending pain pathways to the medulla, midbrain, thalamus and cortex.

Figure 2.6: Neurophysiology of incoming pain. Sensation from peripheral receptors travels along specific pain nerves and is modulated throughout the spinal cord and brain.

The most essential afferent fibers, transmitting nociceptive information are Aδ and c fibers. Spinal reflexes activated by these fibers can guide to removal from a noxious stimulus before pain is perceived by higher structures. Ascending pain pathways consist of
two major anatomical-functional projections which are the sensory-discriminative component to the cerebral cortex and the motivational-affective component to the limbic cortex. Projections to the sensory cortex alert an individual to the existence and anatomic locality of pain. The activation of spinothalamic neurons in the spinal cord can be blocked by descending inhibitory pathways from the midbrain and by sensory Aβ fibers arising in peripheral tissues. These two systems compose the neurologic basis of the gate-control hypothesis.

As per this assumption, pain transmission by spinothalamic neurons can be modulated or gated by the inhibitory activity of other types of large fibers impinging on them. The activation of spinothalamic neurons is also repressed by peripheral Aβ sensory fibers that stimulate the release of enkephalins from spinal cord interneurons. The descending inhibitory pathways occur from periaqueductal gray (PAG) in the mid brain. The medullary neurons comprise serotonergic nerves arising in the nucleus magnus raphae (NMR) and noradrenergic nerves arising in the locus ceruleu (LC). When these nerves free serotonin and norepinephrine in the spinal cord, they decrease dorsal spinal neurons that send out pain impulses to supraspinal sites. Nerve fibers from the PAG also trigger spinal interneurons that release an endogenous opioid peptide, met-enkephalin. The enkephalins act presynaptically to decrease the release of pain transmitters from the central terminations of most significant afferent neurons. They also work on postsynaptic receptors
on spinothalamic tract neurons in the spinal cord to refuse the rostral transmission of the pain signal.

2.6.3 Opioid Peptides and Receptors:

The existence of stereoselective receptors for morphine in brain tissue show the likelihood of an endogenous ligand for these receptors and these finally led to finding of three most important families of endogenous ligand for these receptors and this lastly led to the finding of the three main families of endogenous opioid peptides.

- Enkephalins
- Endorphins
- Dynorphins

Opioid peptides are ensuing from larger precursor molecules encoded by separate genes - proenkephalin, proopiomelanocortin and prodynorphin respectively. Enkephalnergic interneurons in the dorsal horn generate presynaptic inhibition of main afferent neurons and post synaptic inhibition of secondary neurons and post synaptic inhibition of secondary neurons in rising pathways. Endorphin and dynorphins are large peptides. The three opioid receptor types are

\( \mu, \delta, \kappa \) (mu) receptors,

\( \delta, \kappa \) (delta) receptors,

\( \kappa \) (Kappa) receptors
2.6.4 Diagnosis:

There is no approach to report intensity of pain. No test can decide the intensity of pain, no imaging device can give you an idea about pain and no instrument can trace pain accurately. Sometimes, as in the case of headaches, doctors find that the most excellent aid to diagnosis is the patient's own explanation of the type, period, and locality of pain. These descriptions are component of what is called the pain history, taken by the doctor during the preliminary examination of a patient with pain. Doctors, however, do have a number of technologies to discover the cause of pain. Mainly these include: Electro-diagnostic procedures include electromyography (EMG), nerve conduction studies and imaging, especially Magnetic resonance imaging (MRI), provides doctors with images of the body's structures and tissues.

2.6.5 Management of Pain

The aim of pain management is to build up function, enabling individuals to take part in day-to-day activities. Patients and their doctors have different options for the management of pain; some are more doing well than others. Sometimes, relaxation and the use of imagery as a disturbance give relief. The following treatments are among the most ordinary.

Acupuncture involves the use of needles to accurate points on the body. It is component of an ordinary type of healing called traditional Chinese or Oriental medicine.
Cognitive-behavioral therapy includes a broad variety of coping skills and relaxation methods to help get ready for and cope with pain. It is normally used for postoperative pain and the pain of child birth.

Electrical stimulation includes transcutaneous electrical stimulation (TENS), implanted electric nerve stimulation and deep brain or spinal cord stimulation. This is the current extension of age-old practices in which the nerves of muscles are subjected to a multiple of stimuli, including heat or massage. The next techniques each need particular equipment and personnel trained in the specific procedure being used.

TENS includes tiny electrical pulses, provided through the skin to nerve fibers leads to alteration in muscles contractions. This gives short-term pain relief. There is also data that TENS can trigger subsets of peripheral nerve fibers that can block pain transmission at the spinal cord level, in much the similar way that shaking your hand can decrease pain.

Peripheral nerve stimulation uses electrodes positioned surgically on a carefully chosen area of the body. The patient is then capable to deliver an electrical current as required to the affected area, using an antenna and transmitter.

Spinal cord motivation includes electrodes surgically insertion inside the epidural space of the spinal cord. The patient is capable to deliver a pulse of electricity to the spinal cord by a small box-like receiver and an antenna taped to the skin.
Deep brain or intracerebral stimulation is considered an intense treatment and involves surgical stimulation of the brain, usually the thalamus. It is used for a restricted number of conditions, including severe pain, central pain syndrome, cancer pain, phantom limb pain, and other neuropathic pains.

Exercise has come to be an approved part of some doctors' treatment regimens for patients with pain. Because there is a identified link between numerous types of chronic pain and tense, weak muscles, exercise—even light to moderate exercise such as walking or swimming—can contribute to an overall sense of well-being by improving blood and oxygen flow to muscles.

Hypnosis and relaxation are well-known ancillary clinical techniques in the management of acute and chronic pain. The findings from chronic pain studies recommend that hypnotic treatment is consistently better to non-treatment and often as effective but not superior to other viable treatments. There are so many types of drugs used to relieve pain, include Opioid/narcotic/morphine. Next one is Non-opioid/non-narcotic/aspirin like/antipyretic or anti-inflammatory analgesics in these Salicylates, pyrazolone derivatives, indole derivatives, propionic acid derivatives, anthranilic acid derivatives, aryl-acetic derivative, Oxicam derivatives, pyrrolo-pyrrole derivative, sulfonamide derivative, alkanones and last one is analgesic but poor anti-inflammatory activity those are paraaminophenol derivatives, pyrazolone derivatives, and benzoxazocine derivative.123
2.6.6 Herbal Therapies

The following herbal remedies have been well-known to give pain relief:

• Capsaicin is found naturally in cayenne pepper.
• Bromelin reduces inflammation.
• Curcumin reduces inflammation.
• Willow bark reduces inflammation.
• Pine-bark and grape-seed extracts: reduces inflammation.\textsuperscript{126}

2.6.7 Animal Models to Screen Analgesic Activity\textsuperscript{109, 127}:

Central Analgesic Activity

In - Vitro Methods:

1. \textsuperscript{3}H-Naloxone binding assay
2. \textsuperscript{3}H-Dihydomorphine binding to opiate receptors in rat brain
3. \textsuperscript{3}H- Bremazocine binding to opiate receptors in Guinea pig Cerebellum
4. Inhibition of enkephalinase

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
Peripheral Analgesic Activity

1. Writhing tests
2. Pain in inflamed tissue (RANDALL-SELITTO-test)
3. Effect of analgesics on spinal neurons
# Table 2.5: Medicinal Plants Used for the Treatment of Analgesic and Anti-Inflammatory Activity\(^\text{128}\)

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Family</th>
<th>Parts Used</th>
<th>Type of Extract</th>
<th>Experimental Models</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albizia lebbeck</td>
<td>Leguminosae</td>
<td>Bark</td>
<td>Cold extraction of mixture of petroleum ether, ethyl acetate and methanol</td>
<td>Acetic acid induced writhing, radiant heat tail flick method</td>
</tr>
<tr>
<td>Annona squamosa</td>
<td>Annonaceae</td>
<td>Bark</td>
<td>Petroleum ether</td>
<td>Acetic acid induced writhing test, carrageenan induced paw oedema</td>
</tr>
<tr>
<td>Artemisia absinthium</td>
<td>Compositae</td>
<td>Seed, Stem</td>
<td>Methanol extract</td>
<td>Tail immersion method, carrageenan induced paw edema</td>
</tr>
<tr>
<td>Bauhinia racemosa</td>
<td>Caesalpiniaceae</td>
<td>Stem bark</td>
<td>Methanol extract</td>
<td>Acetic acid induced writhing, carrageenan induced paw oedema</td>
</tr>
<tr>
<td>Carissa carandas</td>
<td>Apocynaceae</td>
<td>Root, fruit</td>
<td>Ethanolic extract</td>
<td>Eddy’s hot plate, carrageenan induced rat paw edema, analgesy meter induced pain, cotton pellet induced granuloma</td>
</tr>
<tr>
<td>Cassia sieberiana</td>
<td>Caesalpiniaceae</td>
<td>Root</td>
<td>Aqueous extract</td>
<td>Acid induced writhing, carrageenan induced paw edema</td>
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<td>Daphne retusa</td>
<td>Thymelaeaceae</td>
<td>Bark, stem</td>
<td>Ethanol extract and different fractions (pet. Ether, methylene chloride, ethyl acetate and n-butanol)</td>
<td>Carrageenan induced paw oedema, ear oedema, acetic acid induced writhing, hot plate test</td>
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<td>Diospyros variegata</td>
<td>Ebenaceae</td>
<td>Stem</td>
<td>Hexane extract</td>
<td>Acetic acid induced writhing, formalin test, tail flick method, arachidonic acid and ethyl phenylpropiolate induced rat ear</td>
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<td>Plant Species</td>
<td>Family</td>
<td>Part</td>
<td>Extract Type</td>
<td>Test Model</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------</td>
<td>-----------</td>
<td>-----------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Garcinia hanburyi</em></td>
<td>Guttiferae</td>
<td>Gum resin</td>
<td>Ethyl acetate extract</td>
<td>Ethyl phenylpropiolate induced ear edema</td>
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<tr>
<td><em>Glycine tomentella</em></td>
<td>Leguminosae</td>
<td>Root</td>
<td>Aqueous extract</td>
<td>Acetic acid induced writhing, carrageenan induced paw edema, formalin test</td>
</tr>
<tr>
<td><em>Heracleum persicum</em></td>
<td>Apiaceae</td>
<td>Fruit</td>
<td>Hydroalcoholic extract</td>
<td>Acetic acid induced writhing, carrageenan induced paw edema</td>
</tr>
<tr>
<td><em>Hypericum canariense</em></td>
<td>Clusiaceae</td>
<td>Aerial part</td>
<td>Infusion, methanol extract and fractions (aqueous, butanol and chloroform fractions)</td>
<td>Acetic acid induced writhing, tail flick test, tetradecanoylphorbol acetate induced ear inflammation model</td>
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<td><em>Lactuca sativa</em></td>
<td>Compositae</td>
<td>Seed</td>
<td>Methanol/petroleum ether (70/30 v/v) extract</td>
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<td>Aerial part</td>
<td>Methanol extract</td>
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<td><em>Mahonia owakensis</em></td>
<td>Berberidaceae</td>
<td>Root</td>
<td>Ethanol extract</td>
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<td><em>Margaritaria discoidea</em></td>
<td>Euphorbiaceae</td>
<td>Stem bark</td>
<td>Water extract</td>
<td>Carrageenan and histamine induced paw oedema, acetic acid induced writhing,</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Part</td>
<td>Extract</td>
<td>Test(s)</td>
</tr>
<tr>
<td>------------------</td>
<td>------------------</td>
<td>------------</td>
<td>-------------------</td>
<td>-------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Memecylon edule</td>
<td>Melastomataceae</td>
<td>Leaf</td>
<td>Hexane, ethyl acetate, methanol and 50% methanol fractions</td>
<td>Interleukin production, ethylphenylpropiolate induced edema and the writhing test</td>
</tr>
<tr>
<td>Newbouldia laevis</td>
<td>Bignoniaceae</td>
<td>Flower</td>
<td>Ethanolic extract</td>
<td>Formalin test, acetic acid induced writhing</td>
</tr>
<tr>
<td>Pergularia daemia</td>
<td>Apocynaceae</td>
<td>Root</td>
<td>Ethanolic extract</td>
<td>Eddy’s hot plate, carrageenan induced rat paw edema</td>
</tr>
<tr>
<td>Pfaffia glomerata</td>
<td>Amaranthaceae</td>
<td>Root</td>
<td>Hydroalcoholic extract</td>
<td>Carrageenan induced paw oedema, granulomatous tissue assay, writhing test, hot plate test</td>
</tr>
<tr>
<td>Pogostemon cablin</td>
<td>Lamiaceae</td>
<td>Aerial part, Leaf</td>
<td>Methanol extract</td>
<td>Writhing, formalin test, carr-induced edema test, antioxidant study, tissue COX-2 and TNF-α determination</td>
</tr>
<tr>
<td>Rheedia longifolia</td>
<td>Clusiaceae</td>
<td>Leaf</td>
<td>Aqueous extract</td>
<td>Acetic acid induced writhing, tail flick method, hyperalgesia and pleurisy induced by lipopolysaccharide</td>
</tr>
<tr>
<td>Rivea hypocrateriformis</td>
<td>Convolvulaceae</td>
<td>Leaf</td>
<td>Ethanol extract</td>
<td>Tail flick models, carrageenan induced inflammation</td>
</tr>
<tr>
<td>Saraca indica</td>
<td>Leguminosae</td>
<td>Leaf</td>
<td>Chloroform, Methanol, water extract</td>
<td>Formalin test, tail immersion method</td>
</tr>
<tr>
<td>Smilax china</td>
<td>Liliaceae</td>
<td>Bark</td>
<td>Aqueous extract</td>
<td>Carrageenan induced paw edema, hot plate method</td>
</tr>
<tr>
<td>Species</td>
<td>Family</td>
<td>Part</td>
<td>Extract Method</td>
<td>Tests</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-------------</td>
<td>-----------</td>
<td>---------------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Trichilia connaroides</em></td>
<td>Meliaceae</td>
<td>Leaf</td>
<td>Chloroform extract</td>
<td>Formaline induced paw edema, acetic acid induced writhing, eddy’s hot plate method</td>
</tr>
<tr>
<td><em>Trigonella foenumgraecum</em></td>
<td>Leguminosae</td>
<td>Seed</td>
<td>Water soluble partially purified extract</td>
<td>Acetic acid induced writhing, carrageenan Induced edema</td>
</tr>
<tr>
<td><em>Verbena tenuisecta</em></td>
<td>Verbenaceae</td>
<td>Flower bud</td>
<td>Volatile oil isolated by hydrodistillation</td>
<td>Carrageenan induced paw edema, acetic acid induced writhing, hot plate method</td>
</tr>
<tr>
<td><em>Xanthium strumarium</em></td>
<td>Compositae</td>
<td>Fruit</td>
<td>Ethanol extract</td>
<td>Acetic acid induced writhing, croton oil induced ear edema</td>
</tr>
<tr>
<td><em>Xeromphis spinosa</em></td>
<td>Rubiaceae</td>
<td>Bark</td>
<td>Bark is extracted by ether, ethyl acetate</td>
<td>Carrageenan induced paw edema</td>
</tr>
<tr>
<td><em>Zizyphus lotus</em></td>
<td>Rhamnaceae</td>
<td>Root, bark, leaf</td>
<td>Methanol extract</td>
<td>Carrageenan induced paw edema, Tail flick method</td>
</tr>
</tbody>
</table>
2.7 Hepatoprotective Activity

2.7.1 Anatomy of Liver: \(^{129}\)

Liver is the biggest organ in the body having weight 1400-1600 gm in the males and 1200-1400 gm in females. Right and left lobes are two major anatomical lobes present.

The right lobe is about six times bigger than the size of the left lobe. The right lobe has quadrate lobe on its inferior surface and a caudate lobe on the posterior surface. The right and left lobes are divided anteriorly by a fold of peritoneum called the falciform ligament, inferiorly by the fissure for the ligamentum teres and posteriorly by the fissure for the ligamentum venosum. The porta hepatis is the section on the mediocre surface of the right lobe where blood vessels, lymphatic and common hepatic duct forms the hilum of the liver.

![Liver Anatomy](image)

**Figure 2.7: Liver Anatomy**
2.7.2. Structure:

A firm soft layer of connective tissue called Glisson’s capsule encloses the liver and is incessant with the connective tissue of the porta hepatis forming a cover around the structures in the porta hepatis. The liver has a twin blood supply, the portal vein brings the venous blood from the intestine and spleen and the hepatic artery coming from the coeliac axis supplies arterial blood to the liver. This dual blood supply provides adequate security against infarction in the liver. The portal vein and hepatic artery split into branches to the right and left lobes in the porta. The right and left hepatic ducts also attach in the porta to form the common hepatic duct. The venous drainage from the liver is into the right and left hepatic veins which enter the inferior vena cava. Lymphatics and the nerve fibres accompany the hepatic artery into their branching and finish around the porta hepatis.

Figure 2.8: Structure of Liver Cell
The lobes of liver are made up of several functional units called lobules. A lobule consists of particular epithelial cells called hepatic cells or hepatocytes set in irregular, branching, interconnected plates around the central vein. Rather than capillaries liver has bigger space lined by endothelium called sinusoids through which blood passes. The sinusoids are also to a certain extent lined with stellate reticuloendothelial (Kupffer's) cells which phagocytes worm, bacteria and toxic substances. Bile secreted by hepatic cells enters bile capillaries that drain into small bile ducts. These ducts ultimately combine to form the larger right and left hepatic duct, which unite and go out the liver as the common hepatic duct. Further this common hepatic duct joins the cystic duct from the gall bladder to form the general hepatic duct. The common hepatic duct and pancreatic duct go into the duodenum in a common duct called the hepatopancreatic ampulla.

2.7.3 Functions of liver: 130

2.7.3.1 Secretion and excretion of bile

Bile is to some extent an excretory product and partially a digestive secretion. Each day the hepatic cell secretes 800-1000 ml of bile.

The principle bile pigment is bilirubin. When worm out, red blood cells broken down iron, globins and bilirubin (derived from heam) is free.
2.7.3.2 Metabolic functions

A) Carbohydrate Metabolism

Following a meal, the liver achieves net glucose utilization (eg, for glycogen synthesis and production of metabolic intermediates via glycolysis and the tricarboxylic acid cycle). This occurs as a result of a confluence of a number of effects. First, the levels of substrates such as glucose enlarge. Second, the levels of hormones that affect the quantity and activity of metabolic enzymes change. Thus when blood glucose more; the ratio of insulin to glucagon in the bloodstream is also increases. The net effect is increased glucose consumption by the liver. In times of fasting (low blood glucose) or stress (when higher blood glucose is needed), hormone and substrate levels in the bloodstream drive metabolic pathways of the liver liable for net glucose creation (eg, the pathways of glycogenolysis and gluconeogenesis). As a result, blood glucose levels are raised to, or maintained in, the ordinary range in spite of wide and sudden changes in the rate of glucose input (eg, ingestion and absorption) and output (eg, utilization by tissues) from the bloodstream.

B) Protein Metabolism

Related to its main role in protein metabolism, the liver is a major site for processes of oxidative deamination and transamination. These reactions permit amino groups to be shuffled among molecules in order to create substrates for both carbohydrate metabolism and amino acid synthesis. Similarly, the urea cycle allows nitrogen to be
excreted in the form of urea, which is much less toxic than free amino groups in the form of ammonium ions.

**C) Lipid Metabolism**

The liver is the midpoint of lipid metabolism. It manufactures almost 80% of the cholesterol synthesized in the body from acetyl-CoA via a pathway that connects metabolism of carbohydrates with that of lipids. Moreover, the liver can produce, store and export triglycerides. The liver is also the spot of keto acid production via the pathway of fatty acid oxidation that connects lipid catabolism with activity of the tricarboxylic acid cycle.

In the process of scheming the body's level of cholesterol and triglycerides, the liver assembles, secretes, and takes up a range of lipoprotein particles. A few of these particles (very low-density lipoproteins [VLDL]) serve to dispense lipid to adipose tissue for storage as fat or to other tissues for instant use. In the course of these functions, the structure of VLDL particles is customized by loss of lipid and protein components. The resulting low-density lipoprotein (LDL) particles are then returned to the liver by virtue of their affinity for a definite receptor, the LDL receptor, found on the surface of different cells of the body, including hepatocytes. Other lipoprotein particles (high-density lipoproteins [HDL]) are synthesized and secreted from the liver. They scavenge more cholesterol and triglycerides from other tissues and from the bloodstream, returning them to the liver where they are excreted. Thus, secretion of HDL and
elimination of LDL are both mechanisms by which cholesterol in excess of that required by different tissues is removed from the circulation.

2.7.3.3 Haematological functions (Haematopoeisis and coagulation)

1. Production of fibrinogen, prothrombin, heparin, and other clotting factors VII, VIII, IX, and C.

2. Destruction of erythrocytes. (At the end of their respective life span)

2.7.3.4 Circulatory function

1) Transfer of blood from portal to systemic circulation

2) Blood storage (regulation of blood volume)

2.7.3.5 Detoxification and protective functions

1. Kupffer cells take away foreign bodies from blood (phagocytosis).

2. Detoxication by conjugation, methylation, oxidation and reduction.


2.7.3.6 Drug metabolism

Liver plays a very vital role in biotransformation of drugs. It converts drug molecule from non-polar to polar. Non polar drugs can be conjugated with more polar compounds, which formulate it water soluble for the urinary excretion.
2.7.4. Liver Diseases:

2.7.4.1 Jaundice

Due to enlarged bilirubin level in blood, there is the yellow pigmentation of the skin, mucous membrane and deeper tissues in jaundice. The regular serum bilirubin level is 0.5 to 1.5 mg%. When this is more than 2 mg %, jaundice occurs.

Types and causes of Jaundice

Jaundice is classified into three types which are haemolytic jaundice, hepatocellular jaundice and obstructive jaundice.

a) Haemolytic Jaundice

Haemolytic jaundice is also called prehepatic jaundice. During this, the excretory function of liver is usual. But, there is excessive damage of red blood cells and thus the bilirubin level in blood is increased the liver cells cannot excrete much bilirubin rapidly. So, it accumulates in the blood resulting in jaundice. In this type of jaundice the free bilirubin level increases in blood and increased in creation of urobilinogenin resulting in the excretion of more quantity of urobilinogenin urine. Any situation that causes haemolytic anemia can lead to haemolytic jaundice.

b) Hepatocellular Jaundice

The jaundice due to the damage of liver cells is called hepatocellular or hepatic jaundice. It is also called hepatic cholestatic jaundice. Here, bilirubin is conjugated. But the conjugated bilirubin
cannot be excreted. So, it returns to the blood. The damage of liver cells occurs because of toxic substances (toxic jaundice) or by infection (infective jaundice). Usually liver is affected by virus resulting in hepatitis.

c) **Obstructive Jaundice**

This is otherwise called additional hepatic cholestatic jaundice or post hepatic jaundice. It is due to the impediment of bile flow at any level of biliary system. The bile cannot be poured into small intestine. In this, blood contains more conjugated bilirubin.

2.7.4.2 **Hepatitis**\(^\text{131}\)

Hepatitis is a liver disease distinguishes by swelling and inadequate functioning of liver. Hepatitis may be acute or chronic. In rigorous conditions, it may lead to liver collapse and death.

**Causes and Types**

Hepatitis is caused by viruses, bacteria poisons, autoimmune disease, drug abuse, alcohol, some therapeutic drugs and inheritance from mother during parturition. There is five types of hepatitis which are A, B, C, D and E.

Hepatitis A and E are caused frequently by intake of water and food contaminated with hepatitis virus. In general these two types of hepatitis are not life threatening. Hepatitis B, C and D are caused by sharing needles with contaminated person, accidental prick by infected needle, having unprotected sex with infected person,
inheritance from mother during parturition and blood transfusion from infected donors.

These three forms of hepatitis are severe diseases when compared to hepatitis A and E. Among these, hepatitis B is more general and considered more severe because it may lead to cirrhosis and cancer of liver.

2.7.4.3 Cirrhosis

The inflammation and injure of parenchyma of liver is known as cirrhosis of liver. This may results in degeneration of hepatic cells and dysfunction of liver. Cirrhosis is a diffuse, chronic, necrotic (degenerative) liver disorder characterized by progressive hepatocyte injury followed by regeneration and fibrosis leading to ineffectiveness of lobular architecture, pseudo lobule formation and acquired vascular malformation.

2.7.4.4 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
2.7.4.5 Hepatocellular Carcinoma

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

**Etiology and Pathogenesis**

- Infection with hepatitis B virus.
- Cirrhosis
- Environmental toxins e.g. alpha toxins B produced by Aspergillus flavus.
- Oral contraceptive.

2.7.4.6 Hepatocellular Failure

It may occur due to-

- Ultra structural lesions of hepatocytes e.g. Raye's syndrome.
- Chronic liver diseases e.g. chronic hepatitis, cirrhosis, Wilson's disease.
- Coma

2.7.4.7 Hepatic Encephalopathy

Hepatic Encephalopathy is also called as hepatic coma having characteristic of chronic liver failure. It is a metabolic disorder of the central nervous system and neuromuscular system related with hepatic failure.

2.7.4.8 Portal Hypertension

In this condition, there is improved resistance to portal blood
flow. It may occur in the situation of Portal vein thrombosis, splenomegaly, and cirrhosis.

2.7.5. **Physiology of liver:**

Each second the liver cells go through thousands of complex biochemical reactions that influence all the functions of other organs in the body.

The liver has reserve functional authority and can work successfully when the majority of the hepatocytes are not functioning well. In addition, diseased hepatocytes can in fact redevelop and return to regular function.

![Figure 2.9: Drug Metabolism in Liver](image)

The human body identifies about all drugs as foreign substances (xenobiotics) and subjects them to different chemical processes. This includes chemical transformations to reduce fat solubility and to change biological activity. Even though around all
tissue in the body have some capacity to metabolize chemicals. Liver is the major "metabolic clearing house" for both endogenous chemicals (e.g. cholesterol, steroid hormones, and proteins) and exogenous substances (e.g. drugs). The vital role played by liver in the clearance and transformation of chemicals also makes it susceptible to drug induced injury.

Drug metabolism is normally divided into two phases: phase 1 and phase 2. Phase 1 reaction is considered to get ready a drug for phase 2. However numerous compounds can be metabolized by phase 2 straightforwardly. Phase 1 reaction includes oxidation, reduction, hydrolysis, hydration and lots of other rare chemical reactions. These processes tend to increase water solubility of the drug and can produce metabolites, which are more chemically active and potentially toxic. Most of phase 2 reactions occured in cytosol and includes conjugation with endogenous compounds via transferase enzymes.

A group of enzymes located in the endoplasmic reticulum, known as Cytochrome P-450, is the mostly important family of metabolizing enzymes in the liver. Cytochrome P-450 is the terminal oxidase constituent of an electron transport chain. It is not a single enzyme, somewhat consists of a family of closely related 50 isoforms, six of them metabolize 90% of drugs. There is an incredible diversity of individual P-450 gene products and this heterogeneity allows the liver to carry out oxidation on a vast range of chemicals in phase 1. Three significant characteristics of the P-450 system have roles in toxicities
caused by drug.\textsuperscript{133}

**Table 2.6: Classification of Hepatotoxins and Mechanism of Action of Each Group of Hepatotoxins.\textsuperscript{134, 135}**

<table>
<thead>
<tr>
<th>Category of agents</th>
<th>Mechanism of action</th>
<th>Histologic lesion</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intrinsic Toxicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct</td>
<td>Direct</td>
<td>Necrosis and/or steatosis</td>
<td>CCl\textsubscript{4}, phosphorus</td>
</tr>
<tr>
<td></td>
<td>Physicochemical destruction by peroxidation of hepatocytes.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indirect cytotoxic</td>
<td>Interference with hepatocellular metabolic pathways</td>
<td>Steatosis or necrosis</td>
<td>Ethionine, ethyl alcohol, tetracycline,</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>Interference with bile excretory pathways</td>
<td>Cholestasis duel destruction</td>
<td>Methylenedianiline, anabolic and contraceptive steroids</td>
</tr>
<tr>
<td><strong>Host Idiosyncracy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>Drug allergy</td>
<td>Necrosis or cholestasis</td>
<td>Chlorpromazine, phenytoin, sulfonamides.</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Production of hepatotoxic metabolites</td>
<td>Necrosis or cholestasis</td>
<td>Isoniazid, valproic acid</td>
</tr>
</tbody>
</table>
Table 2.7: Clinical consequences of liver disease

<table>
<thead>
<tr>
<th>Characteristic signs</th>
<th>Hepatic dysfunction :</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Jaundice and cholestasis</td>
</tr>
<tr>
<td></td>
<td>Hypoalbuminemia</td>
</tr>
<tr>
<td></td>
<td>Hyperammonemia</td>
</tr>
<tr>
<td></td>
<td>Hypoglycemia</td>
</tr>
<tr>
<td></td>
<td>Fetor hepaticus</td>
</tr>
<tr>
<td></td>
<td>Palmar erythema</td>
</tr>
<tr>
<td></td>
<td>Spider angiomas</td>
</tr>
<tr>
<td></td>
<td>Hypogonadism</td>
</tr>
<tr>
<td></td>
<td>Gynecomastia</td>
</tr>
<tr>
<td></td>
<td>Weight loss</td>
</tr>
<tr>
<td></td>
<td>Muscle wasting</td>
</tr>
<tr>
<td></td>
<td>Portal hypertension from cirrhosis :</td>
</tr>
<tr>
<td></td>
<td>Ascites</td>
</tr>
<tr>
<td></td>
<td>Splenomegaly</td>
</tr>
<tr>
<td></td>
<td>Hemorrhoids</td>
</tr>
<tr>
<td></td>
<td>Caput medusae-abdominal skin.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Life-threatening complications</th>
<th>Hepatic failure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Multiple organ failure</td>
</tr>
<tr>
<td></td>
<td>Coagulopathy</td>
</tr>
<tr>
<td></td>
<td>Hepatic encephalopathy</td>
</tr>
<tr>
<td></td>
<td>Hepatorenal syndrome</td>
</tr>
<tr>
<td></td>
<td>Portal hypertension from cirrhosis</td>
</tr>
<tr>
<td></td>
<td>Malignancy with chronic disease</td>
</tr>
<tr>
<td></td>
<td>Hepatocellular carcinoma</td>
</tr>
</tbody>
</table>
### Table 2.8: 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 <em>Serum bilirubin</em></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 <em>Serum albumin</em></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><em>Aminopyrine breath test (hepatic demethylation)</em></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 2.9: 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 (C. diptheria, Clostridium botulinus), 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, antidepressants.</td>
</tr>
<tr>
<td>Anti-inflammatory and anti-muscle spasm agents</td>
<td>Cinchopen, colchicine, ibuprofen, salicylates, indomethacin.</td>
</tr>
<tr>
<td>Antimicrobials</td>
<td>Clindamycin, novobiocin, penicillin, tetracycline, sulfonamide,</td>
</tr>
</tbody>
</table>


Antineoplastic

<table>
<thead>
<tr>
<th></th>
<th>amodiaquine, isoniazid, rifampin.</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Asparaginase</td>
<td>L-Asparaginase, azacytidine,</td>
</tr>
<tr>
<td>azacytidine</td>
<td>methotrexate, 6-mercaptopurine,</td>
</tr>
<tr>
<td>methotrexate</td>
<td>chlorambucil, clavacin.</td>
</tr>
</tbody>
</table>

2.7.6 In vivo Models:

A lethal dose or repeated doses of a known hepatotoxin (paracetamol, carbon tetrachloride, thioacetamide, alcohol, tert-butyl hydroperoxide, allylalcohol, etc) are administrated to make liver injure in experimental animals. The test substance is given along with, prior to and/or after the toxin treatment. If the hepatotoxicity is forbidden or reduced by the pre-treatment or after toxin challenge then it is conclude that the test substance is functional.\(^{137-144}\)

Liver damage and healing from damage are observed by finding serum marker enzymes, bilirubin, histopathological changes in the liver and biochemical changes in liver (e.g.: hydroxyproline, lipid etc). When the liver is injured, liver-enzymes such as glutamate pyruvate transaminase (GPT), glutamate oxaloacetate transaminase (GOT) and alkaline phosphatase go into the circulation. An increase in the levels of these marker enzymes in the serum is a sign of liver damage. Other effect of induced liver damage such as decline of prothrombin synthesis giving an extensive prothrombin time and decrease in clearance of certain substances such as bromosulphthalein can be used in the measurement of hepatoprotective plants.
The hepatoprotective effect of a drug against different hepatotoxins differs mainly when the mechanism of action of toxins are different. Therefore, the efficiency of each drug has to be experienced against hepatotoxins, which take action by different mechanisms.

2.7.6.1 Mechanism of paracetamol (PCM) induced hepatotoxicity:

Paracetamol (N-acetyl-p-aminophenol) is a normally used analgesic, antipyretic drug and is safe when used in therapeutic doses. However, more dosage of paracetamol is renowned to be hepatotoxic and nephrotoxic in man and in experimental animals.145 Paracetamol is a straight hepatotoxin i.e. intoxication is dose dependent and reproducible.146 Exposure of animals to high doses produces centrilobular or massive hepatic necrosis followed by congestion and breakdown. The liver necrosis is connected with injure to sub-cellular organelle as well as mitochondria. Thus the drug is used as a typical hepatotoxin to produce hepatic failure experimentally.147

At small doses, about 80% of ingested paracetamol is eliminated frequently as sulfate and glucoronide conjugates previous to oxidation and only 5% is oxidized by hepatic cytochrome P450 (CYP2E1) to a highly reactive and toxic electrophile i.e. N-acetyl-p-benzoquineimine (NAPQI). After over dosage of paracetamol the glucoronidation and sulfation routes turn into saturated and as a consequence, paracetamol is more and more metabolized into NAPQI.148
Semiquinone radical, one-electron reduction metabolite of NAPQI mediates the cytotoxic effects of NAPQI. Formation of these toxic semiquinone radicals is catalyzed by the microsomal cytochrome P450 reductase. These semiquinone radicals, in turn, can bind straightly with cellular macromolecules to create toxicity or on the other hand, the radical can be reoxidized back to their original quinones by donating one electron to molecular oxygen under aerobic situation. This donation of one electron then generates reduced oxygen radical species and hydroxyl radical. Both semiquinone and oxygen radical are recognized to be responsible for cytotoxic effects observed with quinones.

On the additional 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 Glutathione (GSH). Both of these direct two-electron reductions will occur with no any formation of the toxic semiquinone or oxygen radicals and therefore, may give 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 hold back the function of cellular proteins. Adducts creation has been confirmed for a selenium-binding protein, for
microsomal subunit of glutamine. Other mechanism, such as oxidation of pyridine nucleotides and lipid peroxidation may plays important role into cell injure by paracetamol overdose.146

However at high doses of paracetamol NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which outcome in the liver GSH pool reduction and the reactive intermediate reacts with other nucleophilic centers of fundamental molecules in liver cells leading subsequently to hepatotoxicity. As well as, paracetamol is also exposed to directly restrain cellular proliferation, induce oxidative stress, resulting in lipid peroxidation, deplete ATP levels and alter Ca++ homeostasis; all of these changes are measured potentially fatal to the cell.147, 148

![Figure 2.10: Metabolic Pathway of Paracetamol (Acetaminophen)]

2.7.6.2 Mechanism of Thioacetamide Induced Hepatotoxicity:

Thioacetamide was originally used as a fungicide to guard against decay of organs.151
It was shortly recognizable 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 linked with an enlarged extent of lipid peroxidation. The toxicity experienced by the liver during thioacetamide poisoning results from the formation of its metabolite, namely thioacetamide-5-oxide, which is a direct hepatotoxin. Thioacetamide is metabolised by liver CYP 450 2E1, enzymes and sulfoxide derivatives which are apparently accountable for structural proteins and enzyme inactivation.

The thioacetamide-5-oxide is accountable for the alteration in cell permeability, increased intracellular concentration of calcium, rise in nuclear volume and also inhibits mitochondrial activity which causes cell death.

Prominent levels of serum enzymes are indicative of cellular leakage and loss of functional reliability of cell membrane in liver. In-vivo administration of thioacetamide to rats leads to cell death in centrilobular zones both by apoptosis and necrosis. The cellular changes produced by apoptosis happen subsequent a cascade of cell signaling and cascade mediated events and is triggered by two most significant pathways: extrinsic and intrinsic pathway.

The extrinsic pathway implicates death ligands such as Fas ligand, TNFα, TRAIL and their receptors. The intrinsic pathway
involves apoptotic stimuli induced by cytotoxic drugs or oxidative stress which target mitochondria.\textsuperscript{158} This pathway includes the release of cytochrome C from mitochondria to the cytosol, which induces apoptosome complex formation and results in protease procaspase-9 activation and following activation of procaspase-3 through proteolytic cleavage visualized by the reduction of proform level and form of cleavage products. Both the pathway causes caspase-3 commencement and cleavage of restricted set of essential cellular protein, leading to cell dismantlement.

2.7.6.3 Mechanism of carbon cetrachloride (CCl\textsubscript{4}) induced hepatotoxicity:

CCl\textsubscript{4} is an efficient hepatotoxin producing centrilobular hepatic necrosis, which causes liver damage.\textsuperscript{159} CCl\textsubscript{4} induces fatty liver and cell necrosis and have a most important part in inducing triacylglycerol accumulation, depletion of GSH, enlarged lipid peroxidation, membrane damage, and dejection of protein synthesis and failure of enzyme activity.\textsuperscript{160}

It is now usually accepted that the hepatotoxicity of CCl\textsubscript{4} is the effect of reductive dehalogenation, which is catalyzed by cytochrome P450 enzyme and forms the tremendously reactive trichloromethyl free 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
beginning step in a chain of events, which lastly leads to membrane lipid peroxidation, liver injury and to finish as cell necrosis.\textsuperscript{161-164}

CCl\textsubscript{4} is reductively transformed by P450 to the trichloromethyl radical.

\textbf{Figure 2.11: Schematic Representation of Reactive Mechanism of CCl\textsubscript{4} Induced Hepatic Injury}

In current times these substituted fatty acids have been popular to be partially resistant to substitute from endoplasmic reticular phospholipase A\textsubscript{2}. This seems to be result of cross linking of trichloromethyl fatty acid radical, which adds to double bond of other adjacent fatty acids.

\textbf{Figure 2.12: Covalent binding to lipids}

The physiologic importance of this cross-linking on membrane structure and function may be of great significant, mostly 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 CHCl$_3$ and or fatty acid radical. Also 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.$^{165}$

### 2.7.6.4 Mechanism of D-Galactosamine (D-GalN) / Lipopolysaccharide (LPS) induced hepatotoxicity:

D-GalN/LPS induced hepatocellular injury, a deep-rooted 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 leads to necrosis of the liver by uridine triphosphate (UTP) reduction and inhibition of protein synthesis, although D-GalN is regularly used in combination with lipopolysaccharide or tumor necrosis factor. Accumulation of Uridine diphosphate (UDP) sugar nucleotides may contribute to the changes in the rough endoplasmic reticulum and to the problem in the protein metabolism. Additional, extreme galactosamination of membrane structure is thought to be accountable for failure in the activity of ionic pumps. The damage in the calcium pump, with consequent rise in the intracellular calcium is considered to be responsible for cell death. In recent years, apart from the well renowned inhibition of protein synthesis, it has been recommended that reactive oxygen species formed by activated macrophages might be the major cause in D-GalN-induced liver injury. Liver injury induced by D-GalN/LPS
usually reflects disturbances of liver cell metabolism which lead to characteristic changes in the activities of serum enzymes. The problem in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to changed permeability of membranes. In this context, we can view a significant rise in the serum activities of AST, ALT, ALP, LDH and γ-GT which is in accordance with the earlier findings. The action of these enzymes can be used for analysis as indicators of prognosis of the disease because the levels of these marker enzymes are proportional to the degree of damage.

The rise in serum levels of AST and ALT has been credited to the injured structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular injury.\[143\]

2.7.6.5 Mechanism of azathioprine (AZP) induced hepatotoxicity:

The AZP-induced hepatotoxicity created twenty four hours post treatment is renowned by major increments in the activities of both serum Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) and established 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 typical configuration. Moreover, the marked necrobiotic changes in the liver were frequently in the form of degenerated, vacuolated cells and karyolysis or pyknosis of nuclei. Histopathological changes in the liver included 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. A single dose of AZP has been exposed to increase the serum ALT and AST activities twenty four hours post treatment. In hepatocytes Glutathione (GSH) is consumed all over metabolism of AZP to 6-mercaptopurine (6-MP). The mechanism of AZP toxicity to hepatocytes includes reduction of GSH leading to mitochondrial injury with profound depletion of Adenosine tri-phosphate (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 Melanaldehyde (MDA). Therefore it is conceivable that AZP-induced depletion of hepatic GSH and its connected rise of MDA originated as an effect of the AZP-induced elevation of free radicals which in turn speed up lipid peroxidation and cause irreversible cell damage.\textsuperscript{144}

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

Tert-Butyl alcohol is metabolized in hepatocytes by two dissimilar pathways. One includes cytochrome P450; leading to the formation of toxic peroxyl and alkoxyl radicals that begin lipid peroxidation which affect cell integrity and form covalent bonds with cellular molecules, leading in cell death. The second toxicological pathway of t-BHP is a detoxification reaction involving glutathione peroxidase, which gives increase to t-butanol and glutathione disulfide
(GSSG) that in turn alters Ca\(^{2+}\) homeostasis and increases physiological organization of reactive oxygen species (ROS). GSH is widely distributed among living cells and is concerned in several biological functions. It is well-established that GSH acts as a essential intracellular reducing agent for maintenance of antioxidant molecules and the thiol groups on intracellular proteins, namely de Ca\(^{2+}\)ATPase transporter of endoplasmic reticulum. GSH is also the most essential 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 reduction in total glutathione and GSH contents. A significant feature of t-BHP hepatotoxicity is related with its turn down by glutathione peroxidase to the corresponding alcohol, at the expense of GSH which is converted to GSSG. Under harsh exposure to t-BHP, the decrease 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 reduction of GSH, because it scavenges the resulting peroxyl and alkoxyl radicals being oxidised to GSSG. GSH reduction was not proportionally interrelated with GSSG rise and these results can be due to covalent binding of GSH to some electrophilic species produced from t-BHP metabolism or to GSH reaction with protein thiols, protecting them from oxidation. Lipid peroxidation has been recognized as a probable mechanism of cell injury.\(^{145}\)
2.7.6.7 Ethyl alcohol induced hepatotoxicity:

Alcoholic liver disease continues to be a mostly harsh liver disorder all over the world, including India, where alcohol is regularly consumed in the form of country made liquor (CML). A change in liver functions due to alcohol varies from fatty liver to cirrhosis. After its ingestion, ethanol is eagerly absorbed from the gastrointestinal tract. Just 2-10% of that absorbed is eliminated through the kidneys and lungs. The left behind alcohol is oxidized in the body, generally in the liver.\(^{166}\)

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

![Metabolic Pathway of Alcohol](image-url)

**Figure 2.13: Metabolic Pathway of Alcohol\(^ {167}\)**
Alcohol dehydrogenase pathway:

A major pathway for ethanol disposition includes adenosine di phosphate (ADH), an enzyme that catalyzes the modification of ethanol to acetaldehyde. In ADH mediated oxidation of ethanol, hydrogen is transferred from the substrate to the co-factor nicotinamide adenine dinucleotide (NAD), converting it to its reduced form (NADH) and acetaldehyde is produced. The dissociation of the NADH-enzyme complex has been exposed to be a rate restrictive step in this reaction. As a net result, the most important 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 noticeable shift redox potential of the cytosol. This is calculated by changes in the lactate: pyruvate ratio that causes hyperlactacidemia. This change occur because of both decreased utilization and enhanced construction of lactate by the liver.

The changed redox state also alters gluconeogenesis from amino acids and favors hypoglycemia. The enhanced NADH/NAD ratio increases the concentration of glycerophosphate, which favors hepatic triglyceride accumulation by trapping fatty acids. A most essential interaction site of ethanol in the citric acid cycle (in the mitochondria) is beginning with ketoglutarate oxidation. Moreover, the redox change related with ethanol oxidation decreases the hepatic concentration of oxaloacetate, the availability of which monitors the activity of citrate synthetase. The mitochondria will consequently use the hydrogen
equivalents originating from ethanol, rather than from oxidation through the citric acid cycle of two carbon fragments resulting from fatty acids. Thus, fatty acids that typically serve as the most important energy source for the liver are supplanted by ethanol. Depressed fatty acid oxidation by ethanol has been confirmed in, isolated hepatocytes, human liver biopsy tissue and in-vivo. This change results in the deposition of fat in the liver, the early stage of alcoholic liver damage. In other experimental model, chronic alcohol consumption is related with the progression of alcoholic liver injury beyond the fatty liver stage, affecting even protein metabolism.¹⁶⁸

The capability of acetaldehyde to cause lipid peroxidation has been confirmed in isolated perfused livers and has been linked to acetaldehyde oxidation. In count, the binding of acetaldehyde with cysteine, cysteine containing glutathione or both may contribute to the depression of liver glutathione, thereby dropping the scavenging of toxic free radicals by this tripeptide. Rats given ethanol for long periods have noticeably enlarged rates of glutathione turnover in association with improved activity of hepatic gamma-glutamyl transpeptidase. Rigorous glutathione reduction favours lipid peroxidation which can be prohibited or altered in vivo by the administration of methionine, a precursor of cysteine and glutathione. The enhanced activity of microsomal NADPH oxidase after ethanol utilization may result in improved superoxide and hydrogen peroxide creation, thereby hypothetically favouring lipid peroxidation.
In addition, it has been postulated that purine metabolism by means of xanthine oxidase may cause the making of oxygen radicals. Another potential mechanism of cellular damage in acute alcoholic liver disease is creation of free-radicals by neutrophils.\textsuperscript{169}

Although the pathogenesis of early alcoholic liver disease is still mostly anonymous, accumulating proof suggests that endotoxins (lipopolysaccharide (LPS), tumor necrosis factor α (TNF- α) and free radicals are involved. Ethanol raises permeability of the isolated small bowel to endotoxin and elevates circulating endotoxin. This is most probable the original point of a pathophysiologic cascade which causes liver injury. Circulating LPS links with LPS-binding protein (LBP) and the LPS-LBP complex binds to the CD14 receptor of Kupffer cell, the resident liver macrophages are the most important population of the monocyte macrophage lineage. In recent times, 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 free many potent effectors cytokines. Furthermore, in humans, it was in current times shown that promoter polymorphism of the CD14 receptor gene is a hazard factor for liver disease caused by alcohol.

Recently, a study with the constant intragastric feeding model in mice showed that early alcohol-induced liver injury was uncreative in animals lacking TNF- α in bile from rat exposed to ethanol in the Tsukamoto-French model. Free radical signals were diminished over
50% when kupffer cells were damaged by treatment with gadolinium chloride (GdCl₃).¹⁷⁰
Table 2.10: Medicinal Plants Used for the Treatment of Hepatoprotective Activity

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Family</th>
<th>Parts used</th>
<th>Solvent used</th>
<th>Chemical constituents</th>
<th>Screening method</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Amaranthus caudatus</em> Linn</td>
<td>Amaranthaceae</td>
<td>Whole plant</td>
<td>Methanol</td>
<td>Flavonoids, saponins, glycosides</td>
<td>Carbon tetra chloride induced</td>
</tr>
<tr>
<td><em>Anisochilus carnosus</em> Linn</td>
<td>Lamiaceae</td>
<td>Stems</td>
<td>Ethanol</td>
<td>Alkaloids, flavonoids, glycosides</td>
<td>Carbon tetra chloride induced</td>
</tr>
<tr>
<td><em>Asparagus racemosus</em> Linn</td>
<td>Asparagaceae</td>
<td>Roots</td>
<td>Ethanol</td>
<td>Phenols, coumarins</td>
<td>Paracetamol induced</td>
</tr>
<tr>
<td><em>Azima tetracantha</em></td>
<td>Salvadoraceae</td>
<td>Leaves</td>
<td>Ethanol</td>
<td>Flavonoids, triterpenoids</td>
<td>Paracetamol induced</td>
</tr>
<tr>
<td><em>Calotropis procera</em></td>
<td>Asclepidiaceae</td>
<td>Root bark</td>
<td>Methanol</td>
<td>Terpinoidsglycosides, flavonoids</td>
<td>Carbon tetra chloride induced</td>
</tr>
<tr>
<td><em>Cajanus cajan</em> Linn</td>
<td>Leguminosae</td>
<td>Pigeon pea leaf</td>
<td>Ethanol</td>
<td>Flavonoids, stibenes</td>
<td>D-galactosamine</td>
</tr>
<tr>
<td><em>Cajanus scarabaeoides</em> Linn</td>
<td>Fabaceae</td>
<td>Whole plant</td>
<td>n-butanol, ethanol</td>
<td>Flavonoids</td>
<td>Paracetamol induced</td>
</tr>
<tr>
<td><em>Carissa carandas</em> Linn</td>
<td>Apocyanaceae</td>
<td>Root</td>
<td>Ethanol</td>
<td>Alkaloids, tannins, steroids</td>
<td>Carbon tetra chloride induced</td>
</tr>
<tr>
<td><em>Clitoria ternatea</em> Linn</td>
<td>Fabaceae</td>
<td>Leaves</td>
<td>Methanol</td>
<td>Phenolic flavonoids</td>
<td>Paracetamol induced</td>
</tr>
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<td>Cucurbitaceae</td>
<td>Fruit</td>
<td>Pet ether, chloroform, Alcohol</td>
<td>Flavonoids</td>
<td>Carbon tetra chloride induced</td>
</tr>
<tr>
<td><em>Ficus religiosa</em> Linn</td>
<td>Moraceae</td>
<td>Stem bark</td>
<td>Methanol</td>
<td>Glycosides, steroids, tannins</td>
<td>Paracetamol induced</td>
</tr>
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<td>Clusiaceae</td>
<td>Fruit rind</td>
<td>Ethanol</td>
<td>Benzophenones, garcinol</td>
<td>Carbon tetra chloride induced</td>
</tr>
<tr>
<td><strong>Gmelina asiatica</strong> Linn</td>
<td>Verbenaceae</td>
<td>Aerial parts</td>
<td>Ethanol</td>
<td>Flavonoids</td>
<td>Carbon tetra chloride induced</td>
</tr>
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<td><strong>Hyptis suaveolens</strong> Linn</td>
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<td>Flavonoids</td>
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<td>Whole plant</td>
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</tr>
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<td>Ethanol</td>
<td>Spectro photometric method</td>
<td>Carbon tetra chloride induced</td>
</tr>
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<td>Fruit</td>
<td>Ethanol</td>
<td>Saponins, triterpins, steroids</td>
<td>Streptozotocin induced</td>
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<td>Myoporaceae</td>
<td>Leaves</td>
<td>Aqueous</td>
<td>Flavonoids</td>
<td>Profenofos induced</td>
</tr>
<tr>
<td><strong>Myrtus communis</strong> Linn</td>
<td>Myrtaceae</td>
<td>Leaves</td>
<td>Methanol, nbutanol</td>
<td>Flavonoids, terpenoids, steroids</td>
<td>Paracetamol induced</td>
</tr>
<tr>
<td><strong>Solanum nigram</strong></td>
<td>Solanaceae</td>
<td>Fruit</td>
<td>Ethanol</td>
<td>Flavonoids, terpenoids</td>
<td>Carbon tetra chloride induced</td>
</tr>
</tbody>
</table>