CHAPTER – 1

INTRODUCTION AND LITERATURE REVIEW

1.1 INTRODUCTION

Medicinal plants are used to treat illness and diseases for thousands of years. They have gained economical importance because of their application in pharmaceutical, cosmetic, perfumery and food industries. The interest in herbal systems of medicine is growing day-by-day because nature can cure many diseases (Rekha Rajendran et al., 2010).

Medicinal plants are used in treatment of various diseases. *Asparagus racemosus*, *Withania somnifera*, *Glycrrhiza glabra* etc., are used in treatment of anaemia, *Piper longum*, *Adathoda vasica*, *Zingiber officinale* etc., are used in bronchial asthma, *Terminalia chebula*, *Phyllanthus emblica*, *Ricinus communis* etc., are used in Arthritis. *Terminalia chebula*, *phyllanthus emblica*, *Tribulus terrestris* etc., are used in obesity. *Withania somnifera*, *Tribulus terrestris*, *Zingiber officinale* etc., are used in treatment of paralysis, *Piper longum*, *Zingiber officinale*, *cucuma longa*, *Ocimum sanctum* etc., are used to improve blood circulation, *Azadirachta indica*, *Holarrhena antidysenterica*, *Tinospora cordifolia* etc., are used in cancer therapy (Ramar Perumal Samy et al., 2008)

Medicinal plants of commercial significance include poppy, Isabgol, Senna, Cinchona, Ipecac, Belladonna, Ergot, Amla, Chirata, Kalmegh, Safed musli, Ashoka, Ashwagandha, Bael, Shatavari, Tulsi, Brahmi, Chandan, Pippali etc.

Hotspots are areas of exceptional concentration of endemic species. Endemic species (Genera, Families) are restricted in their geographical distribution and do not occur outside these areas. Nearly 25 hotspots have been recognised worldwide (Myers et al., 2000) which harbour 44% of all endemic plant species. Among
these areas, Western Ghats along Srilanka, Eastern Himalayas and Andaman-Nicobar Islands along with Indo-Burma regions are recognized as hotspots of India. Out of the 49,219 plant species, 5150 are endemic to India and distributed into 141 genera under 47 families corresponding to about 30% of the world's recorded flora, which means 30% of the world's recorded flora is endemic to India. Of these endemic species, 3,500 are found in the Himalayas and adjoining regions and 1600 in the Western Ghats alone.

There are seven endemic medicinal plants in Tirumala hills of Chittoor district of Andhra Pradesh. They are *Boswellia ovalifoliata*, *Cycas beddomei*, *Pimpinella tirupatiensis*, *Petrocarpus santalinus*, *Shorea thumbuggaia*, *Syzygium alternifolium*, *Terminalia pallida* (Shaik Abdul Latheef et al., 2008).

If endemic plants are not protected, they may become extinct. The Govt of India has recognized some plant species which need to be conserved, they include: *Azadirachta indica*, *Aegle marmelos*, *Andrographis paniculata*, *Asparagus racemosus*, *Bauhinia vahlii*, *Emblica officinalis*, *Holarrhena antidysenterica*, *Gymnema sylvestre*, *Litsea glutinosa*, *Mallotus philippenensis*, *Pterocarpus marsupium*, *Soymida febrifuga*, *Strychnos potatorum*, *Sapindus emarginatus*, *Strychnos nux-vomica*, *Terminalia bellerica*, *Terminalia chebula* (Ved and Goraya, 2007). The Government of India has mounted a programme of Vanaspathi Van Project to promote Indian System of Medicine and for development of medicinal plants in degraded forests.

Diabetes is one of the major culprits responsible in degrading the health of a person in this stressful life. During world war-II when insulin was not available in many countries, search was made for a substitute for insulin from plant sources. Moreover drugs used in Type-2 have a number of limitations as they produce severe adverse effects and high rate of secondary failure (Xie et al., 2002). Many plant species in folk medicine were used for their hypoglycemic properties and therefore used to treat diabetes (Cragg et al., 1997). Some of the plants with anti diabetic activity include *Allium cepa*, *Coccinia indica*, *Ficus glomerata*, 14
Gymnema sylvestre, Momordica charantia, Pterocapus marsupium, Rauwolfia serpentina, Syzygium cumini (Kong, 2003). Plants with proven hypoglycemic effects were found to contain compounds like terpenoids (Manikako et al., 1997) glycosides (Das et al., 1996) (Grove et al., 2002), Alkaloids (Ivoora et al., 1989) and saponins (Routhu et al., 2005) etc.

Liver is major functional organ in the body and its diseases are serious health problems which are encountered very commonly in present era. The cause for these problems may be drugs, chemicals, alcohol, environmental pollution etc. Conventional medical therapy for many common liver disorders, including non alcoholic fatty liver disease and viral hepatitis has limited efficacy and potentially life threatening side effects. Various medicinal plants are used in traditional medicine for their hepato protective effects. The most commonly used medicinal plants for management of liver diseases include Phyllanthus spp (Euphorbiaceae) Silybum marianum, Glycerrhiza glabra (Kong, 2003) etc.

Plants are considered to be biosynthetic innovatives, which produce primary and secondary metabolites. Many primary metabolites like carbohydrates, proteins and lipids and secondary metabolites like glycosides, alkaloids, tannins, volatile oils etc., which have therapeutic effects in human beings and animals are obtained from these solar powered biosynthetic laboratories. Secondary metabolites have been shown to alter biological processes which may reduce the risk of chronic diseases in humans. An impressive number of modern drugs have been isolated from natural sources. Many of these isolations were based on the uses of the agents in traditional medicines (Saini et al., 2009). Modern research has made it possible to isolate and identify active constituents from the extracts and to verify their therapeutic activity and specify dose-response relationship. Inspite of developments in synthetic chemistry, higher plants are still a source of the medicinal compounds. With a view to explore traditional medicines and to investigate their scientific application, an endemic medicinal plant Soymida febrifuga Adr. Juss, which has been used as a traditional folklore medicine, was selected for the present work.
1.2 REVIEW OF LITERATURE

1.2.1 Review of literature of *Soymida febrifuga*

*Soymida febrifuga* A.Juss. belongs to family Meliaceae (Chopra *et al.*, 1956). It is an indigenous lofty deciduous medicinal tree endemic to India (Anonymous wealth of India, 1952).

The nomenclatural history of *soymida febrifuga* A. Juss (Meliaceae) is studied by several botanists. The specific name was published in 1793 by Roxburgh, The generic name was given by Mirbel and Cassini in the year 1830, and the combination in 1832 (Mabberley, 1982).

**Classification (Zipcodezoo.com)**

- Domain : Eukaryota
- Kingdom : Plantae
- Sub-Kingdom : Viridaeplantae
- Phylum : Tracheophyta
- Sub-phylum : Euphyllophytina
- Infrafylum : Radiatopses
- Class : Magnoliopsida
- Sub-class : Rosidae
- Super order : Rutanae
- Order : Rutales
- Sub order : Melineae
- Family : Meliaceae
- Subfamily : Solanoideae
- Tribe : Solaneae
Fig. 1: Soymida febrifuga
Vernacular names (Kirtikar, 2003)

<table>
<thead>
<tr>
<th>Region</th>
<th>Names</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bengal</td>
<td>Rohan, Rohira,</td>
</tr>
<tr>
<td>Bombay</td>
<td>Rohing</td>
</tr>
<tr>
<td>Central Provinces</td>
<td>Rohini, Rohun,</td>
</tr>
<tr>
<td>Deccan</td>
<td>Rohunna, Rouen, Ruhina,</td>
</tr>
<tr>
<td>English</td>
<td>Bastard cedar, Indian red wood, Rohan tree.</td>
</tr>
<tr>
<td>Gond</td>
<td>Somi</td>
</tr>
<tr>
<td>Gujarati</td>
<td>Rohani, Rohina</td>
</tr>
<tr>
<td>Hindi</td>
<td>Rakat rohan, Rohunna.</td>
</tr>
<tr>
<td>Khond</td>
<td>Soniangi</td>
</tr>
<tr>
<td>Lambadi</td>
<td>Ronero</td>
</tr>
<tr>
<td>Marathi</td>
<td>Potar</td>
</tr>
<tr>
<td>Merwara</td>
<td>Rohan</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Agniruha, Atiruh, Chandravallabha, Kashamansi</td>
</tr>
<tr>
<td></td>
<td>Lomakarani, Mahamansi, Mansarohini</td>
</tr>
<tr>
<td></td>
<td>Praharavalli, Patranga, Suloma, Vasa, Vikasha,</td>
</tr>
<tr>
<td></td>
<td>Viravalli, Vritta</td>
</tr>
<tr>
<td>Tamil</td>
<td>Sem, Somadanam, Sombu, Sumi, Surakkali</td>
</tr>
<tr>
<td>Telugu</td>
<td>Sevamanu, Somi, Somida, Somili</td>
</tr>
<tr>
<td>Urdu</td>
<td>Rohan</td>
</tr>
<tr>
<td>Uriya</td>
<td>Karwi, Sohan, Sonhan, Suam</td>
</tr>
</tbody>
</table>

**Distribution:** It grows well in dry forests of W. Peninsula. Extending northwards to Merwara, the Mirzapur hills and Chota Nagpur, Ceylon, dry deciduous forests of India, A.P. It is found in N. Circars from Ganjam to Godavari, on laterite hills and in the forests of Deccan from Kurnool to Mysore and hills of Chingleput. It is found in Rajamundry, Tirupathi, Pakhal regions of A.P. Grows well on lime soils, black cotton soils, and dry stony hills. It is also found in dry forests of Kerala, Gujarat, U.P. Bihar, Ceylon, Karnataka, Madhyapradesh, Maharastra, Orissa, Rajasthan, Tamilnadu, Srilanka (Kirtikar, 2003).
Morphological Description: It is a tall tree. Leaves 23-45 cm long, crowded towards the ends of branches. Leaflets 3-6 pairs, opposite, elliptic (or) oblong, obtuse, glabrous, penni nerved, nerves are numerous and conspicuous beneath. Base is rounded in equilateral i.e. the lower side generally extending further down the peliole than the upper. Petioles are red in colour. Flowers in large terminal (or) axillary divaricately branched panicles often equaling the leaves, they are greenish white and appear in February-May. Sepals 5, rotund, margins membranous, slightly lacerate, petals 5, obovate, 6mm long, clawed, often notched at the apex. Staminal tube is about half as long as the petals, slightly urceolate, anthers, attached by the middle of the back. Ovary is glabrous, stigma large, discoid. Ovary is supplied only by carpellary ventrals. Ovules show attachment to parietal placentae. Fruit ripens in May-June. The capsules are 2.5-6.3 cm long, 5-celled and 5 valves separating from dessipinents which remain attached to thick spongy axis. Numerous seeds in each cell, flat, winged at both ends, with a soft felly covering. Bark slightly red, scale like (Murthy et al., 1976)

Pharmacognostic review of Soymida febrifuga:

Literature Survey revealed the Pharmacognostic study of leaves of Soymida febrifuga. Transverse section of leaves (Fig.2) showed the presence of

Upper Epidermis: Single layered, covered with thick cuticle. The cells are thin walled, polygonal in shape and large in size.

Palisade tissue: It is arranged in two layers, first layer is large in length. This layer is followed by spongy parenchyma and intracellular space. Colouring matter is seen in palisade cells & spongy parenchyma. Crystals of calcium oxalate are also present in spongy tissue. Vascular strands are present.

Lower Epidermis: It is single layered. The cells are similar in shape to upper epidermal cells but small in size. Mid rib is very prominent on both surfaces. It has ridges which are composed of collenchymatous cells. Vascular bundles occupy
the middle region. This is surrounded by sclerenchymatous cells (3-5 layers). Ground space of midrib is filled up by spongy parenchyma. The xylem vessels and sclerenchymatous fibres are lignified. Starch is absent (Attarde et al., 2010).

**Powder microscopic study:** Scleride cells are fibre like with tapering ends. The walls are thick having wide lumens and pits are canal like and simple. They are lignified. Here also druses and prismatic type crystals are found. Druses are scattered in powder, prismatic crystals occur is strands.

![Fig. 2: T.S of Soymida febrifuga leaf](image)

**Transverse section of bark:**

Literature review of Pharmacognostic study of bark revealed the following features (Fig.3, 4). T.S of bark consists of outer periderm. Innerbark consists of phloem tissue arranged in two zones i.e., outer zone of crushed and collapsed phloem fibres and inner zone of intact phloem. Phloem rays are present in outer zone. Tannis and Calcium oxalate are mainly deposited in collapsed phloem. Phloem rays are narrow is the region of intact phloem. Calcium oxalate crystals are abundant in collapsed phloem tissue. They are very large and occur in two forms i.e., druses and prismatic crystals (Malarkodi Velraj et al., 2009).
Fig. 3. Section of Bark

PhP = Phloem parenchyma, PhR = phloem ray, ST = Sieve tube

Fig. 4. Section of Bark

CPh = Collapsed Phloem, NCPh = Non Collapsed Phloem, PhR = Phloem ray
**Microscopy of wood:** Crystals are very important diagnostic aids in identification of woods. Cross, tangential and radial, sections of wood of *Soymida febrifuga* were studied without staining the sections. Crystals were identified both in procumbent ray cells and upright ray cells. Upright ray cells that have crystals are idioblasts, while the procumbent cells containing crystals are normal in size. Crystals are found in non-chambered parenchyma (Krishna Negi *et al.*, 2003).

**Phytochemistry:** Various phytochemicals were isolated and characterized by C-NMR, IR, H-NMR, and LC-MS. They include lupeol, sitosterol, methylangolensate (Lakshmi *et al.*, 1987), deoxyandirobin, from wood and bark, Quercetin - 3- O-L-rhamnoside, and 3-O-rutinoside from leaves (Nair *et al.*, 1975), two new tetranor triterpenoids (I and II), from bark.

![Structural formulas of compounds I and II](image)

Another new tetranortriterpenoid – febrifugin was isolated from heartwood and its structure was elucidated (Murali Krishna *et al.*, 1978).

![Structure of febrifugin](image)

Naringenin, myricetin, dihydromyricetin and quercetin were isolated from heartwood (Rao *et al.*, 1979). Methyl angolensate and Luteolin -7-Oglucoside (Adesida *et al.*, 1971) were isolated from callus cultures of root.
Naringenin

Dihydromyricetin

Quercetin

Methyl angolensate

Luteolin – 7-oglucoside

Deoxyandirobin

Luteolin

β-Sitosterol
Traditional uses: *Soymida febrifuga* bark extracts are used in treatment of rheumatoid arthritis (Kirtikar, 1984), asthma and good for ulcers (Kirtikar, 2003)).

The decoction of the bark has bitter resin used in vaginal infections, rheumatic pains and stomach pains. Bark is used as anti-cancer remedy, used in wounds, dental diseases, uterine bleeding and haemorrhage (Ambaye et al., 1971). It is used as an acrid, refrigerant, Antihelmintic agent, aphrodisiac, laxative, good for sore throat, removes vata and cures tridosha fevers, cough, asthma (Yoganarasimhan et al., 1996). Removes blood impurities, good for ulcers, leprosy, dysentery and it has anti inflammatory activity. The bark is used in intermittent fevers and general debility, in advanced stages of dysentery and diarrhea. It is a good anti malarial like cinchona. It has antimicrobial activity.

The bark is astringent to bowels and used in fevers in Yunani medicine, decoction is a good substitute for Oak-bark used for gargles, vaginal infections & enemas. The bark is a bitter tonic. A decoction of bark 1 in 20 was given in one ounce doses three times a day in cases of malarial fever (Kirtikar, 2003). Decoction of bark is used in tongue sores, fixing loose teeth, gum infection. The bark is crushed and used with water and administered in cough (Murthy et al., 2001).

Medicinal Properties and uses:

*Invitro* antiplasmodial activity on *Plasmodium falciparum* was studied. Of 80 analysed ethanolic extracts belonging to 47 species, 31 produced significant effect. Among them *Casearia elliptica, Holarrhena pubescens, Pongamia pinnata, Soymida febrifuga* and *Plumbago zeylanica* showed significant activity (Simonsen et al., 2001).

The tree bark of mansarohini extract showed dose dependant inhibition of rat paw oedema. The anti inflammatory activity was comparable to NSAID’s used like naproxen, ibuprofen, and piroxicam. It is devoid of ulcerogenic properties. It was confirmed to be a potential anti inflammatory agent (Diwan et al., 1993).
Anti oxidant and antimicrobial properties of hexane, methanol and aqueous extracts of *Soymida febrifuga* leaf were evaluated for anti oxidant activity and anti microbial activity. Aqueous extracts were reported to have highest antioxidant activity and total phenolic content than hexane extract (Boreddy Srinivas Reddy *et al.*, 2008).

Methyl angolensate, which is a natural tetranortriterpenoid isolated from *Soymida febrifuga* root calluses was responsible for anti cancer activity. It was active against T-cell leukemia, and chronic myelogenous leukemia (Kishore *et al.*, 2008).

The activity of various extracts was tested for protective action on Hepg₂ cells. All the extracts were reported to have *invitro* antioxidant and protective activity. The crude leaf and bark extracts were also found to have *invitro* hepatoprotective and hypoglycemic activities. The extracts were found to have mild *invivo* hypoglycemic activity (Boreddy Srinivas Reddy *et al.*, 2008).

**Reasons for taking up the present work**

The National Medicinal plants board (NMPB) in its recent investigation listed *Soymida febrifuga* under most vulnerable group of species that need immediate management focus.

Extensive survey of literature revealed the medicinal importance of *Soymida febrifuga* A.Juss. Few of its traditional medicinal uses include the use of bark in rheumatic pains, vaginal infections, and helminth infestation. It has aphrodisiac, anti inflammatory, anti microbial, anti malarial actions like cinchona.

Phytochemical reports on this plant revealed that *Soymida febrifuga* contains glycosides, tetranortriterpenoids, alkaloids, steroids etc.,
They are the phytoconstituents present in medicinal plants which are responsible for antidiabetic, anti hepatotoxic, aldose reductase inhibiting activities (Kohda et al., 1989) (Suryanarayana et al., 2004) (Rodriguez et al., 2003) (El-Domiaty et al., 2009) (Boreddy Srinivas Reddy et al., 2008).

Eventhough there are few reports on the plant, a systematic investigation has not be taken up. In view of the above claims and facts, the present investigation was undertaken.

This thesis embodies a systematic investigation, *invitro* studies of crude extracts, *invitro* and *invivo* studies of few fractions obtained from methanolic extract of the bark of *Soymida febrifuga*.

**Objective of work:**

1. Collection of plant material
2. Preparation of extracts using different solvents.
3. Preliminary Phytochemical screening
4. *Invitro* methods for evaluation of:
   a. Anti oxidant activity
   b. 5-Lipoxygenase inhibiting activity
   c. Anti cancer activity
   d. Antihelmintic activity
5. Fractionation
6. *Invivo* experiments
   a. Acute Toxicity Testing
   b. Screening for hypoglycemic and anti hyperglycemic activities of isolated fractions
      i. Assessment of Hypoglycemic activity in euglycemic rats.
      ii. Assessment of glucose tolerance in normal healthy rats
iv. Effect of fractions on biochemical parameters in sub-acute study in Alloxan induced Type – II diabetes in rats.
c. Evaluation of aldose reductase inhibitory activity of isolated fractions
   i. *Invitro* method using rat lens homogenate.
   ii. *Invitro* method using rat kidney homogenate.
   iii. *Invivo* method using galactosemia induced Wistar-Albino rats.
   iv. HPLC analysis.
d. Screening for Hepato protective and Anti hepatotoxic activities in carbon tetrachloride and drug induced Hepatotoxicity in rat models.
   i. Assessment of hepatoprotective effect of the fractions in CCl4 induced acute hepatotoxicity in rats.
   ii. Assessment of anti hepatotoxic effect of the fractions in CCl4 induced acute hepatotoxicity in rats.
   iii. Effect of fractions on Barbiturate induced sleeping time in rats.
   iv. Assessment of Hepatoprotective activity of fractions on drug induced hepatotoxicity in rats.
   v. Histopathological studies.
e. Statistical analysis of data
1.2.2 Free radicals:

Generation of Free Radicals

The living cell during several metabolic pathways generates reactive oxygen species (ROS) and reactive nitrogen species (RNS). Pathophysiological conditions enhance the generation of ROS and RNS and lead to oxidative stress. The generation of ROS begins with the rapid uptake of oxygen and activation of NADPH oxidase and the production of the super oxide free radical.

\[ 2\text{O}_2 + \text{NADPH} \xrightarrow{\text{oxidase}} 2\text{O}_2^\bullet + \text{NADP} + \text{H}^+ \]

ROS can also be generated through the Fenton (A) reactions (I) and Haber-Weiss reaction (II) (Knight et al., 1999). Transition metals catalyse decomposition of Hydrogen peroxide generating hydroxyl radicals and other ROS.

I. \[ \text{H}_2\text{O}_2 + \text{Fe}^{2+} \longrightarrow \text{Fe}^{3+} + \text{OH}^\bullet + \text{OH}^- \]

It has two reactions

II. \[ \text{H}_2\text{O}_2 + \text{OH}^\bullet \longrightarrow \text{H}_2\text{O} + \text{O}_2^\cdot + \text{H}^+ \]

\[ \text{H}_2\text{O}_2 + \text{O}_2^\cdot \longrightarrow \text{O}_2 + \text{OH}^- + \text{OH}^\bullet \]

The free radical nitric oxide (NO), which is known as endothelium-derived relaxation factor (EDRF), is formed from arginine by nitric oxide synthase (NOS).

\[ \text{L-arg} + \text{O}_2 + \text{NADPH} \xrightarrow{\text{NOS}} \text{NO}^\bullet + \text{Citrulline} \]

\[ \text{NO}^\bullet + \text{O}_2 \xrightarrow{\text{NOS}} \text{ONOO}^- \text{(Peroxynitrite)} \]

Peroxynitrite is a very strong oxidant, which reacts with aromatic amino acid residues to form nitrotyrosine, which can lead to enzyme inactivation. To escape ROS, RNS and lipid peroxidation dependant injury, biological structures have protective machinery in the form of endogenous antioxidants. Among different endogenous antioxidants, super oxide dismutase (SOD), reduced glutathione (GSH), catalase and glutathione peroxidase (GPX) are important for counteracting oxidative stress.
Lipid Peroxidation

Lipid peroxidation is a complex process which occurs in aerobic cells. Interaction of molecular oxygen with unsaturated fatty acids results in lipid peroxidation. This produces and propagates the Lipid radical ($L^\bullet$), uptake of $O_2$, generation of lipid alkoxy ($LO^\bullet$), lipid peroxyl radicals ($LOO^\bullet$), rearrangement of double bonds, lipid hydroperoxide ($LOOH$) as well as a number of degradation products. The disturbance of balance between the free radicals (lipid radicals) and antioxidants leads to oxidative stress.

Role of free radicals in diabetes and hepatic problems:

Free radicals are continually produced in the body as a result of normal metabolic processes and interaction with chemical stimuli. They play an important role in causation and complications of diabetes (Mohammed et al., 1999) (Wolff et al., 1993). They are formed disproportionately in diabetes by glucose autoxidation, polyol pathway and non-enzymatic glycation of proteins (Soto et al., 2003) (Rosen et al., 2001).

Abnormally high levels of free radicals and decreased antioxidant defence mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation and development of complications of Diabetes mellitus. Oxidative stress is currently suggested as mechanism underlying diabetes and its related complications (Hallivell et al., 1997). Implication of oxidative stress in pathogenesis of diabetes is suggested not only by oxidative-radical generation, but also due to non-enzymatic protein glycosylation, auto-oxidation of glucose (Mullarkey et al., 1990) impaired glutathione metabolism (McLennan et al., 1991) alteration in antioxidant enzymes, lipid peroxides formation (Baynes et al., 1991) and decreased ascorbicacid levels. The products of lipid peroxidation are also associated with atherosclerosis and brain damage.
Several forms of liver damage have been claimed to involve free radicals, among which are those produced by haloalkanes, azodyes, alkyl nitrosamines, paracetamol, and ethanol. Free radicals covalently bind to cell structures resulting in their functional modification. This ultimately results in lipid peroxidation where in lipid free radicals as well as other non-radical toxic products are generated, this plays very important role in development of liver damage (Diazani et al., 1994). Free radicals can damage cellular macromolecules and therefore, may participate in hepato cellular injury when produced in excess. Free radical initiated peroxidation may play a role in hepatic fibrogenesis through an effect of aldehydic peroxidation products on kupffer cells and lipocytes.

**Antioxidants**

Compounds which generate toxic oxygen species (or) free radicals are referred to as pro-oxidants. The compounds which dispose the free radicals (or) toxic oxygen species are called as antioxidants. Normally there will be a balance between pro-oxidant and Anti-oxidant in a cell. When the production of oxygen species is more (or) when antioxidant levels decrease, oxidative stress results. This is a consequence of either increased oxidant generation (Node et al., 1997) (or) decrease in antioxidant protection (Yasmin et al., 1997) (or) failure to repair oxidative damage. Free radicals are responsible for a number of problems associated with eye like cataract (Hualei et al., 2003), muscular retinopathy, glaucoma; excess free radicals attack DNA (leading to cancer), blood vessels, (causing cardiovascular diseases). They are implicated in Arthritis, Strokes, Diabetes, Liver diseases, Alzheimer’s disease, Aging, Ischemic dementia (CiddiVeeresham et al., 2006) Experimental and clinical evidence indicates hypercholesterolemia is associated with enhanced oxidative stress. Oxygen free radicals, such as O$_2$ and F$_2$ isoprostanes, have been found to be elevated in the arteries of hypercholesterolemic animals (or) in urine of patients with high serum cholesterol, respectively (Sanguini et al., 2002). Antioxidants act as radical scavengers, hydrogen donors, peroxide decomposers, electron donors, enzyme inhibitors, singlet oxygen quenchers, synergistic and metal chelating agents.
Plants also need to protect themselves from free radical damage, so they have evolved many different classes of phytochemicals to do so. The pigments in the barks, seeds, leaves, fruits and flowers are very active antioxidants because of the presence of phytochemicals including plant phenolics such as phenylpropanoids, flavonoids and coumarins; polyphenolics like proanthocyanidins and tannins; phytosterols, carotenoids, chlorophyll derivatives (pigments); essential oils; flavolignans, gums and resins.

Some of the examples of natural antioxidants are: Silybin, Dihydro quercetin, Catechin, Spermine, Mansonone, Ferulic acid, Chromo-saponin, Emblican A and B, Punigluconin, Pedunculagin, Curcumin, Gallic acid, and Bengalenoside. Antioxidant herbal formulation available in the market is Brahma rasayana, Geriforte, Abana and HD-3, MAK-4 (Tablets) MAK-5 (Paste) and Reatival.

**Antioxidant Defense System (ADS):**

Antioxidant defense system (ADS) against oxidative stress is composed of several lines, and antioxidants are classified into four categories based on their function.

1. Preventive antioxidants, which suppress formation of free radicals.
2. Radical scavenging antioxidants, which suppress chain initiation and or/breaking chain propagation reactions.
3. Repair and de novo antioxidants and
4. Adaption, where the signal for the production and actions of free radicals induces formation and transport of the appropriate antioxidant to the right site (Dukic, 2001).
1.2.3 Lipoxygenase and 5- Lipoxygenase inhibitory activity:

Arachidonic acid is 5, 8, 11, 14 - eicosatetraenoic acid, a 20-carbon unsaturated fatty acid. It is the source of eicosanoids namely Prostaglandins, thromboxanes, leukotrienes. Free arachidonic acid can be metabolized by either cyclooxygenases (or) lipoxygenases. Cyclooxygenases are COX -1, COX-2. They initiate biosynthesis of prostaglandins and thromboxanes. Lipoxygenases initiate biosynthesis of Leukotrienes, the lipoxins and other compounds.

Lipoxygenases are soluble enzymes found in the cytosol. They are mainly concentrated in lungs, platelets, mast cells and WBC. 5-Lipoxygenase is the main enzyme among these lipoxygenases. On cell activation, this enzyme translocates to the cell membrane where it becomes associated with a protein termed 5-lipoxygenase activating protein (FLAP) which is necessary for Leukotriene synthesis in intact cells. Next step is addition of hydroperoxy group to C5 in Arachidonic acid. This finally results in synthesis of LTA₄. This is converted enzymatically into LTB₄, which is a pre cursor of different cysteiny l leukotrienes ie LTC₄, LTD₄, LTE₄, and LTF₄.

Cysteiny l – Leukotrienes: The have actions mainly on respiratory and cardiovascular systems.

- The respiratory system: potent spasmogens, causing dose-related contraction of human bronchiolar muscle *in vitro*. LTC₄, LTD₄, LTE₄ can increase the mucus secretion.

- Cardiovascular system: Small doses of LTC₄ and LTD₄ given by IV, cause rapid, short-lived fall in B.P, by subcutaneous route they are equipotent to histamine in causing wheal and flare. Given topically in nose LTD₄ increases nasal blood flow, and increases local vascular permeability.

- LTB₄ is found in many inflammatory conditions like rheumatoid arthritis, psoriasis, ulcerative colitis. Cysteiny l leukotrienes are found in sputum of
persons with chronic bronchitis. On antigen challenge, they are released from samples of human asthmatic lung in vitro and into nasal lavage fluid invivo in subjects with allergic rhinitis. Cysteinyl leukotriene receptor antagonists currently in use as antiasthmatics are Zarfirlukast and Montelukast. (Rang, 2006).

5-Lipoxygenase inhibitors act on lipoxygenase enzyme and therefore interfere with the synthesis of cysteinyl leukotrienes. So, they have a very important role to play in treatment of asthma and as anti-inflammatory agents.

1.2.4 Cancer:

Cancer is uncontrolled multiplication of cells. Cancer cells have four main characteristics that distinguish them from normal cells. They are:

- Uncontrolled proliferation
- Dedifferentiation and loss of function
- Invasiveness
- Metastasis

A normal cell becomes a cancer cell due to mutation in its DNA. Not only genetic change but epigenetic factors like hormonal action, co-carcinogen, tumour promoters, are not themselves cancer producing but will increase the likelihood that the genetic mutations will result in cancer. The two main categories of genetic change that lead to cancer are:

- The activation of proto-oncogenes to oncogenes.
- The inactivation of tumour suppressor genes.

**Activation of proto-oncogenes to oncogenes:** Proto oncogenes are normal genes. Their function is to control cell division, apoptosis and differentiation. They can be converted to oncogenes by the action of viral (or) by action of carcinogen.
**Inactivation of tumour suppressor genes:** Normal cells have genes that suppress a malignant change. They are tumour suppressor genes (antioncogenes). These genes undergo mutations and result in many different cancers.

**The cell cycle** (Fig.5): This is an ordered sequence of events consisting of various phases (Rang, 2006).

![Cell cycle diagram](image)

- **M** $\rightarrow$ This is a phase of mitosis
- **G\(_1\)** $\rightarrow$ this is an intermediate phase between mitosis and synthetic phase (S). During this phase the cell prepares for DNA synthesis
- **S** $\rightarrow$ This is the phase of DNA synthesis
- **G\(_2\)** $\rightarrow$ This is a gap between S and M phases. During this phase the cell is preparing for mitosis to give rise to two daughter cells.
- **Go** $\rightarrow$ This is a quiescent phase, in an adult most cells do not constantly divide. They spend most of the time in this phase. Quiescent cells can enter G\(_1\) by chemical stimuli.

Different category of free radicals are generated when different types of foods undergo oxidation. These free radicals play important role in cancer, aging, neurological diseases, atherosclerosis etc. (Bagchi and Puri, 1998). Cancer is the largest cause of mortality in the world. Annually about 3500 per a million population world wide are killed by cancer. A number of chemopreventive agents
are used to cure cancer but cause severe side effects (Kathiresan et al., 2006). There is an urgent need for less toxic and more effective anticancer drugs.

Medicinal plants possessing anticancer activity include Androgrophis paniculata, *Azadirachta indica*, *Camellia sinensis*, *Citrus limon*, *Ipomea batata* (Govind Pandey and Madhuri, 2009), *Catharanthus roseus*, *Raphanus sativaus*, *Aglacia sylvestre* (Cragg and Newmann, 2005), *Dysoxylum binectariferum*. Hook. (Kelland et al., 2001). Plant extracts promote host resistance against infection by restabilizing body equilibrium and conditioning body tissues. Therefore there is a broad scope for anticancer agents from plant sources (Govind Pandey and Madhuri, 2009).

**1.2.5 Helminthiasis:**

World Health organization estimates that a staggering 2 billion people are having parasitic worm infestations (www.who.int). Helminth diseases can contribute to prevalence of anaemia, eosinophilia, and pneumonia. This is highly prevalent in third world countries (Dhar et al., 1982) due to poor management practices. Helminth infection of the human body occurs with parasitic worms such as roundworms, pinworms etc. The worms usually only involve the intestinal tract but sometimes may invade other organs. The type and severity of symptoms is determined by the type of worm and the part of the body infected.

Helminth diseases are becoming resistant day-by-day to the currently available Antihelmintic drugs (Coles et al., 1997). Traditional system of medicine reports the efficacy of natural products in eliminating helminths. Medicinal plants are considered to be a rich source of Antihelmintics (Lewis et al., 1977).

The active constituents present in the plant extracts ie, steroids, saponins tannins, flavonoids, terpenoids, have Antihelmintic activity (Dipak Raut et al., 2009) (Sawarkar et al., 2011). Antihelmintics act by disruption of neuromuscular physiology, or blockade of energy metabolism, or by disrupting reproductive system which is highly efficient in these parasites (Geary et al., 1992). Tannins
produce Antihelmintic activity by binding to free protein in the gastrointestinal tract of the host animal or glycoprotein on the cuticle of the parasite. Phenolic compounds (tannins are polyphenolic compounds) by uncoupling oxidative phosphorylation hinder the energy production in helminth parasites (www.sanquer.ac) (Athnasiaduo et al., 2001).

**Symptoms of Helminthiasis**

Abdominal pain, Diarrhea, Fever, Fatigue, Enlarged liver, Enlarged spleen, Cough, Eosinophilia, Asymptomatic gastrointestinal inflammation, Malabsorption, Bowel obstruction, Anaemia, Dehydration, Bloody diarrhea, Skin symptoms, Chestpain, Vomiting, Constipation, Weightloss, Distended abdomen, Itchyskin, Malaise, Headache, Itchy anus, Neurological problems, Irritability.

**Models used to test Antihelmintic activity** (Vidyarthi, 1995) (Mali et al., 2004)

- Pheretima posthuma: (Indian earth worm)
- Haemonchus contortus
- Ascaridia galli

**1.2.6 Diabetes mellitus:**

Diabetes mellitus is a chronic metabolic disorder. This is one of the world’s oldest known Diseases. Egyptian physicians, who described a disease, associated with “the passage of much urine”, recognized diabetes mellitus as early as 1500 B.C. The term “diabetes” (the Greek word for siphon) was coined by Greek physician Artaeus around 2 A.D. The adjective “mellitus” a latin word was added by Willis in 1674, whic meanh means honey (Paranjape et al., 1993). Diabetes mellitus is a group of metabolic disorders characterized by chronic hyperglycemia caused by Insulin deficiency and /or Insulin resistance (Rang, 2006). The metabolic disturbance involves the disturbance in the metabolism of fats, proteins and carbohydrates, caused by insulin deprivation and possibly abnormally high amounts of glucagon and other counter regulating hormones such as
sympathomimetic amines and corticosteroids. This occurs due to deficient insulin secretion and also to factors opposing the tissue effects of insulin or both.

Since the modern world is full of stress, the incidence of diabetes is on increasing trend. In 1997, diabetes prevalence was introduced as a “basic health indicator” for member states by the WHO, which estimated in 1995 that the number of people with diabetes in the world would reach 300 million by 2025 (King et al., 1998). As per literature reports Asians are more prone to diabetes compared to others perhaps because of their food habits of taking carbohydrate rich diet (Habib et al., 2005). Dietary habits and a sedentary lifestyle causes obesity and it has been established that the risk for diabetes increases when the body mass index (BMI) for Asians crosses 23, though by WHO standards, a BMI of 25-29 is overweight and above 30 is obesity.

The prevalence of the disease in India in adults was found to be 2.4% in rural and 4-11.6% in urban dwellers. High frequencies of impaired glucose tolerance shown by those studies, ranging from 3.6-9.1%, indicate potential for further rise in prevalence of Diabetes mellitus in the coming decades. It is projected that India will become the World capital for Diabetes mellitus within a span of ten years (Park, 2000).

**Types of Diabetes Mellitus**

- Insulin dependent diabetes mellitus (IDDM)
- Non - Insulin dependent diabetes mellitus (NIDDM)
- Malnutrition related diabetes mellitus (MRDM)
- Gestational diabetes mellitus (GDM)
- Impaired Glucose Tolerance (IGT) and Impaired Fasting Glucose (IFG)
- Other types associated with certain disease conditions

**Insulin dependent diabetes mellitus (IDDM) / Type-1**: Type-1 also described as juvenile-onset or ketosis prone diabetes, accounts for about 6-10% of known
diabetic population. It is an autoimmune disease of the pancreas, characterized by total destruction of pancreatic β-cells, which causes decreased insulin secretion. Insulin is a peptide hormone comprising of 51 amino acid residues in two polypeptide chains, which are attached to each other by Disulphide Bridge, which acts as a key that opens the doors of the cells to allow glucose to enter. This type of diabetes generally develops during childhood or puberty, usually seen in individuals less than 30yrs of age. It is lethal unless promptly diagnosed and treated.

The glucose absorbed during a meal is not metabolized at the normal rate and therefore accumulates in the blood (hyperglycemia) to be excreted in the urine (glycosuria). Glucose in the urine causes osmotic diuresis, leading to increase urine production (polyuria). Stimulation of protein breakdown to provide amino acids for gluconeogenesis results in muscle wasting and weight loss.

**Non - Insulin dependent diabetes mellitus (NIDDM) / Type-II**

This is much more common than IDDM and accounts to 90% of diabetic patients. Type-2 diabetes (non-insulin dependent diabetes mellitus (NIDDM) usually affects adults older than 45 yrs. The etiology of Type-2 diabetes mellitus (non-insulin- dependent diabetes mellitus, NIDDM) is even less clearly understood. Two factors have been identified:

1) **Impaired insulin release**

Basal secretion of insulin is often normal, but the rapid release of insulin following a meal is greatly impaired, resulting in failure of normal handling of a carbohydrate load. In most patients, some level of insulin secretion is maintained, therefore, ketoacidosis doesn’t occur. In these patients, insulin secretion can be stimulated by drugs such as sulfonylureas. Exogenous insulin is therefore not essential in treatment. It also has been suggested that inheritance of a defective pattern of insulin secretion is responsible for the
familial tendency of diabetes. The genetic factor is very strong in Type-II diabetes, with a history of diabetes present in about 50 % of first degree relatives.

2) **Insulin resistance**

A defect in the tissue response to insulin is believed to play a major role. This phenomenon is called insulin resistance and is caused by defective insulin receptors on the target cells. Insulin resistance occurs in association with obesity and pregnancy. In normal individuals who become obese or pregnant, the β cells secrete increased amounts of insulin to compensate. Patients who have genetic susceptibility to diabetes cannot compensate because of their inherent defect in insulin secretion. Thus, Type-2 diabetes is frequently precipitated by obesity and pregnancy. In a few patients with extreme insulin resistance, antibodies against the receptors have been demonstrated in plasma. These antibodies are mostly of the IgG class and act against the insulin receptors, causing a decrease in number of insulin receptors and defective binding of insulin to receptors.

The classic symptoms in patients with Type-2 Diabetes Mellitus (Non insulin-dependent diabetes mellitus, NIDDM) are glycosuria, proteinuria, postprandial hyperglycemia, microaneurysms, and possibly retinal exudates.

- **Malnutrition related diabetes mellitus (MRDM):** It is a relatively new class of diabetes found in tropical developing countries in patients who are grossly under weight, and who have a history of malnutrition in childhood. Increased consumption of foods containing cyanogenetic glycosides, [Ex: Wild cherry bark (Prunasin), Mustard (Sinigrin), Bitter almond (Amygdalin), Linseed (Linamarin) etc] resulting in pancreatic damage may be the cause for this condition.
• **Gestational diabetes mellitus (GDM):** It is common in about 2-5% of all pregnancies. This diabetes only occurs during pregnancy, but generally symptoms disappear within 6 weeks of delivery. It occurs more frequently in women who have a family history of Type-2 diabetes and about 20-50% of these women go on to develop Type-2 diabetes.

• **Impaired Glucose Tolerance (IGT) and Impaired Fasting Glucose (IFG):** IGT/ IFG describes a state of intermediate “at risk” group between diabetes and normality. This group is defined as having fasting plasma glucose levels > 100mg/dl (5.6mmol/L) but < 126mg/dl (7.0 mmol/L) or 2hr values in the oral glucose tolerance test of > 140mg/dl (7.8 mmol/L) but < 200mg/dl(11.1 mmol/L). Patients with IFG and/or IGT are now referred to as having “pre-diabetes” indicating the relatively high risk for development of diabetes in these patients.

• **Other types associated with certain disease conditions:** Occur as part of related disorders that include diabetes as one of their symptoms. They make up less than 2% of diabetes cases and include defects of the pancreatic cells, defects in insulin action, diseases of the pancreas and kidneys, drug or chemical interactions with the body, infections and other genetic syndromes such as hemochromatosis.

Other specific types of diabetes mellitus include maturity-onset diabetes of the young (MODY), diabetes due to mutant insulin, diabetes due to mutant insulin receptors, diabetes mellitus associated with a mutation of mitochondrial DNA and obese Type II patients (Irfan and Atiya, 2005).

**Risk factors for the development of Diabetes Mellitus**

- Family History of diabetes
- Obesity (>20% over desired body weight)
- Age > 45 yrs
• Sedentary life style
• Some ethnic groups (Particularly Africans and native Americans)
• Gestational Diabetes
• High Blood pressure (>140/90mm of Hg)
• High blood levels of Triglycerides (>250mg/dl)
• Low HDL cholesterol level (<35mg/dl).

Complications

• **Short-term effects**: Short term effects such as blurred vision, polyuria, increase in incidence of urinary tract infections, fatigue, or drowsiness may impair the quality of life of patient untreated or poorly treated for diabetes. Two life-threatening short term complications that necessitate prompt medical intervention are diabetic ketoacidosis and non ketotic hyperosmolar syndrome.

• **Long-term effects**: Although the short-term metabolic effects of hyperglycemia are life threatening and necessitate prompt attention, the long-term effects are insidious and often unnoticed. However, the prolonged effects are serious, very often debilitating, and if untreated, life-threatening over the long-term.

A long-term complication of diabetes includes

- Retinopathy with potential loss of vision.
- Nephropathy leading to renal failure.
- Peripheral neuropathy with risk of foot ulcers, amputations, and charcot joints
- Autonomic neuropathy causing gastrointestinal, genitourinary, ardiovascular symptoms and sexual dysfunctions.
The deleterious pathway responsible for the complications is the production of high concentrations of advanced glycosylation end products (AGE) and sorbitol (Eric et al., 1995).

**Diagnosis of Diabetes Mellitus**

There are various tests to diagnose Diabetes mellitus. The most commonly used tests are

- **A random (casual) plasma glucose test:** Glucose levels more than or equal to 200mg/dl with classic symptoms of diabetes mellitus, including polydipsia, polyuria, polyphagia and weight loss.

- **Fasting plasma glucose test (FPG):** It is fast, economical and commonly used test. Blood is drawn from the patients after an overnight fast. (Fasting is defined as no calorie intake for at least 8hrs).

**Table 1: Fasting plasma glucose levels**

<table>
<thead>
<tr>
<th>Category</th>
<th>FPG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt; 126</td>
</tr>
<tr>
<td>Impaired fasting glucose tolerance (IFG)</td>
<td>126-140</td>
</tr>
<tr>
<td>Diabetic</td>
<td>&gt; 140</td>
</tr>
</tbody>
</table>
- **Oral glucose tolerance test (OGTT):** It measures the ability of the patients to handle a glucose load over a period of time.

**Table 2: Oral glucose tolerance values**

<table>
<thead>
<tr>
<th>Category</th>
<th>FPG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>&lt; 140</td>
</tr>
<tr>
<td>Impaired glucose tolerance (IGT)</td>
<td>140-199</td>
</tr>
<tr>
<td>Diabetic</td>
<td>&gt; 200</td>
</tr>
</tbody>
</table>

FPG = Fasting plasma glucose levels

Patients with IFG and IGT are referred to as having “pre-diabetes” indicating relatively high risk for development of diabetes.

- **Glycosylated Haemoglobin (HbA1c):**
  Haemoglobin glycosylation occurs when Hb is exposed to ambient glucose concentration in the blood. When higher concentrations of blood glucose are present, the % of HbA1c increases because RBCs are freely permeable to glucose. Measurement of glycosylated Hb fraction gives an integrated picture of the average blood glucose concentration over the last 60-90 days.

**Table 3: HbA1c test values**

<table>
<thead>
<tr>
<th>Category</th>
<th>HbA1c</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>4-6</td>
</tr>
<tr>
<td>Excellent control</td>
<td>&lt; 7</td>
</tr>
<tr>
<td>Good control</td>
<td>7-8</td>
</tr>
<tr>
<td>Fair control</td>
<td>8-9</td>
</tr>
<tr>
<td>Poor control</td>
<td>&gt;10</td>
</tr>
<tr>
<td>Diabetic</td>
<td>&gt;20</td>
</tr>
</tbody>
</table>
Therapy of Diabetes Mellitus

All patients with diabetes should receive healthy living advice. This includes advice on appropriate physical activity and lifestyle modification, particularly smoking cessation and healthy eating.

I. Dietary therapy

a) Carbohydrate: The blood glucose level is closely affected by carbohydrate intake. Daily intake should be kept fairly constant, (and the amount given should be kept fairly constant) and the amount given should be appropriate to the level of physical activity.

b) Fat: Since there is an increased risk of death from coronary artery disease in diabetes, it is wise to restrict saturated fats and to substitute unsaturated fat. Fat intake is generally targeted to be less than 30% of total calories and cholesterol intake less than 300 mg/day.

c) Fiber: Dietary fiber has two useful properties. First, it is physically bulky and increases satiety. Second, fiber delays the digestion and absorption of complex carbohydrates, thereby minimizing hyperglycemia (Baily and Day, 1989).

II. Physical activity (exercise)

A carefully planned and consistent program of physical activity enhances glucose uptake to cells, thereby reducing the blood glucose level. The physical activity plan should be consistent with regards to frequency (daily, or at least 3-4 days per week), intensity, and duration (Roger and Clive, 1996).
III. Pharmacotherapy

Currently, six categories of FDA – approved medication for treating diabetes are available. Based on the mode of administration, they are grouped into two i.e. Insulin and Oral hypoglycemic agents viz., Sulfonylureas, Biguanides, α-Glucosidase inhibitors, Thiazolidinediones, Meglitinides. All the categories of medicines can treat patients with Type-2 diabetes effectively, but insulin is the only diabetic medication needed for those with Type-1 disease (Roach et al., 1999).

**Insulin:** Modern therapy of IDDM began with the discovery of the involvement of the pancreas in diabetes by Von Mering and Minkowski in 1889, and the demonstration by Bang and Best in 1921 that an extract of beef pancreas could successfully lower blood glucose levels in pancreatectomized dogs. Their use of a pancreatic extract in a human diabetic in 1922 marked the first use of the pancreatic antidiabetic principle, insulin, in the treatment of diabetes mellitus. Several different preparations of bovine, porcine, and human insulin are now available, including lente or long-acting forms, and are given as injections which represent the current standard of therapy for IDDM.

Insulin is a polypeptide drug which would be subject to digestion in the stomach and small intestine if taken orally. Preparations for nasal and rectal administration have been developed, but low biological efficacy restricts their use. Insulin can be combined with protease inhibitors for administration in the ileum and ascending colon. However, the oral route is the most practical for patients. This has been achieved by encapsulation of insulin in liposomes, impervious polymer films, or in polyalkyl cyanoacrylate nanocapsules which can pass through the intestinal epithelium.
Classification of insulin’s

1. Short and rapid acting insulin’s:
   a) Insulin aspart (HUMALOG)
   b) Insulin lispro (NOVOLOG)

These analogues begin to work within 5-15 min of injection, achieve peak activity in about 60-90 min and have duration of action approximately 4 h. These characteristics allow patients to administer rapid acting insulin’s 30 to 45 min before meals, providing more flexibility in scheduling meal times and better control of postprandial glucose levels.

2. Intermediate acting insulin’s
   a) Neutral Protamine Hagedorn (NPH) insulin (Isophane insulin suspension).
   b) Lente insulin (Insulin zinc suspension).

These analogues begin to work 1-2 h after injection, achieve peak activity in about 6-12 h and have duration of action 18-24 h. In patients with type-2 diabetes, these are helpful to normalize fasting blood glucose given once or twice a day before breakfast.

3. Long acting insulin analogues:

Ultra lente insulin (extended insulin zinc suspension). It is the long acting insulin formulation that has a modest peak at 10 h, and duration of action 18-20 h. Glargine is a long-acting insulin analogue that has flat, peak less profile of activity that lasts for more than 24 h.
4. Premixed insulin formulations

These are providing more convenience and greater accuracy for patients because the patient does not need to mix them. Formulations currently available for use are:

a) NPH and regular insulin 70/30 mixtures.
b) NPH and regular insulin 50/50 mixture
c) Insulin lispro mixture (75/25).

Oral Hypoglycemic Agents

Therapy of NIDDM involves modifications of lifestyle and diet, an exercise regimen, and use of oral hypoglycemic agents. They include:

a) Sulphonyl ureas: e.g.: Tolbutamide, glibenclamide, gliclazide, glipizide, etc. These are derived chemically from the sulfonamides. These drugs act, by binding to ATP-sensitive potassium channel receptors on the pancreatic cell surface, thereby reduce potassium conductance and depolarize the membrane which results in stimulation of calcium ion influx through voltage dependent calcium channels, raise intracellular concentration of calcium ions which induces the secretion or exocytosis of insulin. Their main side effects are hypoglycemia, weight gain, constipation and gastrointestinal discomfort.

b) Biguanides: eg: Metformin, Phenformin. These drugs act by stimulation of intracellular glucose catabolism especially via anaerobic pathways. The later process produces lactic acid cleared mainly by liver where some of it is converted back to glucose. It has two fold mechanisms 1. It enhances peripheral muscle glucose uptake and utilization and 2. Inhibits glucose release from liver. These do not produce weight gain and may actually result in weight loss, making it particularly useful in obese NIDDM patients. The major adverse effect is lactic acidosis.
c) **α -Glucosidase inhibitors**: eg: Acarbose, miglitol. These drugs act by reducing the absorption of glucose from the diet and inhibit the terminal step of carbohydrate digestion at the brush border of the intestinal epithelium which causes a delay in carbohydrate absorption. The unique effect of these agents is to lower postprandial glucose and there by improve glycemic control in all patients without increasing the risk for weight gain (or) hypoglycemic events. The major adverse effects are GI intolerance, abdominal pain and flatulence (Agarwal *et al.*, 1996) (Leboviz, 1997).

d) **Thiazolidine diones (TZD)**: eg: Pioglitazone, Rosiglitazone. These agents are ligands of peroxisome-proliferator activated receptors gamma (PPAR γ) and are associated with slow improvement in glycemic control over weeks to months in parallel with improvement in insulin sensitivity. Use of these agents is some times associated with weight gain and fluid retention. The major side effect is gastrointestinal (GI) back pain.

e) **Short acting insulinotropic agents (or) Meglitinide agents**: eg: Repaglinide, Nateglinide. These agents are insulin secretagogues, act by binding to the K-receptors on β-cells in pancreas and closes ATP-dependent potassium channels, like sulphonylureas, but have a shorter onset and duration of action. It has the potential to control postprandial glucose excursions with low risk for hypoglycemia than sulphonylureas. The major adverse effects are sinus tachycardia, hypothermia, loss of consciousness, blurred vision, uncontrolled yawning. From this brief overview of diabetes classification and modern therapy, it can be seen that current methods of treatment for all types of diabetes mellitus fail to achieve the ideals of normoglycemia and the prevention of diabetic complications. Promising fields of research such as pancreatic transplants offer little hope to the majority of the world’s diabetics, for whom such procedures will be too expensive and difficult to obtain. Therefore, there is a clear need for alternate sources of both oral and parenteral antidiabetic drugs and alternate strategies for diabetes therapy.
A brief account on plants with anti-diabetic potential

Since ancient times, plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them. The ethno botanical information reports about 800 plants that may possess anti-diabetic potential (Chattopadhyay et al., 1992). Several such herbs have shown anti-diabetic activity when assessed using presently available experimental techniques (Alarcon-Aguilara et al., 1998). Wide arrays of plant derived active principles representing numerous chemical compounds have demonstrated activity consistent with their possible use in the treatment of NIDDM (Saifi et al., 1971). Among these are alkaloids, glycosides, polysaccharides, peptidoglycans, hypoglycans, steroids, carbohydrates, glycopeptides, terpenoids, and amino acids. Even the discovery of widely used hypoglycemic drug, metformin came from the traditional approach of using Galega officinalis. Thus, plants are a potential source of anti-diabetic drugs. The details of some of the plants reported to possess antidiabetic activity are summarized in Table 4.
Table 4: Plant extracts/ constituents with antidiabetic activity:

<table>
<thead>
<tr>
<th>Plant Name-Family</th>
<th>Name of the extract/ plant part/ constituents</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aegle marmelos</em> -Rutaceae</td>
<td>Alcoholic extract of Leaves and fruits - Alkaloid-aegelin, aegelinin, tannins, coumarin (marmesin) (Ivoora <em>et al.</em>, 1989).</td>
</tr>
<tr>
<td><em>Aloe vera</em> -Lilliaceae</td>
<td>Alcoholic extract of Leaves - Quercetin, rutin, emodin, Chrysophanic acid (Das <em>et al.</em>, 1996).</td>
</tr>
<tr>
<td><em>Azadirachta indica</em> -Meliaceae</td>
<td>Alcoholic extract of Leaves- Nimbolide (Yu <em>et al.</em>, 2003).</td>
</tr>
<tr>
<td><em>Beeta vulgaris</em> var.cicla -Chenopodiaceae</td>
<td>Aqueous extracts of roots- Betavulgarosides II, III&amp; IV (Murali Krishna <em>et al.</em>, 2008).</td>
</tr>
<tr>
<td><em>Cassia kleinii</em> -Fabaceae</td>
<td>Ethanolic extract of leaves - Terpenoids, coumarins and saponins (Somani <em>et al.</em>, 2005).</td>
</tr>
<tr>
<td><em>Catharanthus roseus</em> -Apocyanaceae</td>
<td>Alcoholic extract of leaves - Ajmalicine (Babu <em>et al.</em>, 2003).</td>
</tr>
<tr>
<td><em>Cuminum nigrum</em> -Apiaceae</td>
<td>Alcoholic extract of seeds – Flavonoids (Badole <em>et al.</em>, 2006).</td>
</tr>
<tr>
<td><em>Gymnema sylvestre</em> -Asclepiadaceae</td>
<td>Alcoholic extract of Leaves - Saponin-Gymnemic acid IV, gymnemocides (Routhu <em>et al.</em>, 2005).</td>
</tr>
<tr>
<td><em>Momordica charantia</em> -Cucurbitaceae</td>
<td>Aqueous extract of Fruit Juice – Alkaloid Momordin -1, Glycoside-Oleonolic acid (Groove <em>et al.</em>, 2002).</td>
</tr>
</tbody>
</table>
The details of some of the marketed herbal Anti-diabetic formulations are given in Table 5.

### Table 5: Some of the marketed herbal Anti-diabetic formulations

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Company</th>
<th>Ingredients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabecon Tablets</td>
<td>Himalaya</td>
<td>Gymnema sylvestre, Pterocarpus marsupium, Aloe vera, Ocimum sanctum, Syzygium cumini, Swertia chirata, Momordica charantia, Tinospora cordifolia, Curcuma longa, Triphala churna etc.</td>
</tr>
<tr>
<td>Bitter gourd Powder</td>
<td>Garry and Sun natural Remedies</td>
<td>Bitter gourd (Momordica charantia)</td>
</tr>
<tr>
<td>Pancreatic tonic 180 cp</td>
<td>Ayurvedic herbal supplement</td>
<td>Gymnema sylvestre, Pterocarpus marsupium, Momordica charantia, Syzygium cumini, Trigonella foenum graecum, Azadirachta indica, Aegle marmelos etc.</td>
</tr>
<tr>
<td>Gurmar Powder</td>
<td>Garry and Sun natural Remedies</td>
<td>Gurmar (Gymnema sylvestre)</td>
</tr>
<tr>
<td>Diabeta Capsule</td>
<td>Ayurvedic Herbal Health Products</td>
<td>Catharanthus roseus, Curcuma longa, Acacia arabica, Zingiber officinale, Syzygium cumini, Pterocarpus marsupium, Tinospora cordifolia, etc.</td>
</tr>
</tbody>
</table>
1.2.7 Aldose reductase and galactosemia

Diabetes mellitus is usually irreversible while it allows the patient to have reasonably normal life style, its complications results in a considerably reduced life expectancy (Eric et al., 1995). Diabetic complications are more dangerous than diabetes itself. According to a report from the US National Health and Nutrition Examination Survey 1999-2004, nearly 60% of patients with Diabetes have more than one complications caused by long standing diabetes. Moreover, diabetic complications are leading causes of morbidity and death in diabetic patients. There is therefore growing interest in drugs that alleviate the various symptoms of diabetic complications (Jung et al., 2008). Aldose reductase enzyme inhibition is an attractive strategy, for the prevention of complications resulted by the increased blood hexose levels. Long standing diabetes can lead to neuropathy, nephropathy, and micro angiopathy which mainly cause retinal and macro vascular diseases.

Aldose-Reductase (AR) Enzyme

Aldose reductase is a cytosolic enzyme and is a small monomeric protein composed of 315 aminoacid residues (Carper et al., 1989). The primary structure of AR was first determined on rat lens aldose reductase, it demonstrated high similarities to another NADPH-dependent oxidoreductase, human liver aldehyde reductase and to ρ-crystallin, a major structural component of the lens of frog Rana pipiens (Tamarev et al., 1984). The degree of similarity clearly suggests that these proteins belong to the same family, namely Aldo-Keto reductase super family, with related structures and evolutionary origins. AR considered as a typical enzyme of this superfamily.
Distribution

Aldose reductase is present in most of the mammalian cells, although the distribution of the enzyme is not uniform among tissues (Nishimura, 1998). In human tissues, it is abundant in the epithelial cells lining the collecting tubules in the renal medulla (Cao et al., 1998). Kidney is one of the richest tissue sources of AR; the enzyme is localized in the medullary portion from which quantities of the enzyme are isolated for biochemical studies (Aida et al., 2000). Additionally, AR is also found in many other tissues such as seminal vesicles, retina, lens and muscle (Cao et al., 1998).

Physiological Functions

It is suggested that the enzyme might function physiologically as a general housekeeping enzyme under normal conditions (Mansour, 2007).

➢ Osmoregulatory role:

AR converts glucose to sorbitol. This sorbitol is one of the organic osmolytes that balance the osmotic pressure of extracellular NaCl, fluctuating in accordance with urine osmolality. These findings therefore suggest the osmoregulatory role of AR in the renal homeostasis (Burg, 1995)

➢ Metabolic role

AR distribution among tissues unaffected by extracellular osmotic stress suggests an alternate metabolic role. In addition, the marked hydrophobic nature of the active site is unusual for an enzyme thought to be involved in the metabolism of aldo-sugars (Mansour, 2007)
➤ **Detoxification role**

AR reduces lipid peroxidation-derived aldehydes as well as their glutathione conjugates (Srivastava et al., 1998). Several structurally different phospholipids, aldehydes are efficiently reduced by this enzyme, suggesting that it may be an important component of mechanisms that remove and detoxify these aldehydes when they are generated in oxidized lipids. It could be concluded that AR fulfills a role as oxidative defence protein. The enzyme may also act as an extra-hepatic detoxification enzyme in various tissues.

Thus, the significance of AR in the Polyol-Pathway may be quite limited under non-diabetic conditions. It provides an osmolyte sorbitol in the renal medulla and supplies fructose as an energy source of sperm in the seminal vesicle (Mansour, 2007). AR is the first and rate-limiting enzyme of the polyol-pathway (Fig 6).

**Pathogenesis**

The AR mediated pathogenesis is dependent on chronically elevated ambient hexose levels as in Diabetes mellitus [DM] and Galactosemia (Petrash, 2004).
Diabetes Mellitus - Secondary complications

Patients with Type-1 (insulin-dependent) or Type-2 (insulin-independent) diabetes develop secondary complications, the risk of which is related to the duration of diabetes and the degree of glycemic control (Turner, 1995). The four most common secondary complications of diabetes are Macrovascular disease, Nephropathy, Retinopathy and Neuropathy (Mansour, 2007).

(a) Factors responsible for glucose toxicity: Organ damage can be triggered by both extracellular and intracellular hyperglycemia.

- Increased extracellular glucose leads to advanced glycation end products [AGEs] formation, due to non-enzymatic glycosylation of proteins, which interact with the Receptor for AGE [RAGE] on the plasma membrane and promote the production of reactive oxygen species [ROS]. Due to the production of these ROS, cells of the kidneys, eye and nervous system undergo cell- and organ-specific phenotypic changes, which are mediated by a range of signaling pathways and transcription factors (Fig. 7).

**Fig7. Effects of Increased Extracellular Glucose**

Extracellular Glucose ↑

↓

Non-enzymatic Glycosylation of Proteins

↓

Advanced Glycation End products (AGEs)

↓

Interaction with Receptor for AGE (RAGE)

↓

Reactive Oxygen Species (ROS)

↓

Range of signaling pathways and transcription factors

↓

Cell and Organ specific phenotypic change
Increased intracellular glucose drives mitochondrial activity, increases the activity of protein kinase C [PKC], and NADPH oxidase and promotes increased flux through the polyol pathway, all of which have many effects on cellular metabolism and phenotype (Fig. 8).

**Fig8. Effects of Increased Intracellular Glucose**

AR in glucose toxicity

Under normoglycemic (euglycemic) conditions, polyol-pathway accounts for approximately 3% of glucose utilization but under hyperglycemic event, the elevated glucose level enhances the activity of AR by increasing the glucose flux through polyol-pathway (more than 30% glucose is metabolized by this pathway). The induction of osmotic stress from the excess intracellular accumulation of sugar alcohol (polyol) can lead to altered membrane permeability and subsequently to biochemical changes that result in the initiation of cellular lesion (polyol osmotic theory) (Yue et al., 1989) (Bhatnagar et al., 1992).
Diabetic retinopathy

Diabetes is the leading cause of new cases of blindness among adults aged 20-74 years. Diabetic retinopathy takes many years to develop, and almost all patients with Type-1 and Type-2 diabetes exhibit some lesions after 20 years of disease. Nevertheless, only in a fraction of patients disease will progress to visual impairments (Oishi et al., 2002). Diabetic retinopathy is characterized by a range of retinal lesions and abnormalities that indicate vascular damage (capillary micro-aneurysms, capillary degeneration, increased vascular permeability and new vessel formation) and death or dysfunction of the neural retina (‘cotton wool spots’, alteration in retinal electrophysiology, and loss of colour or hue discrimination) (Roy, 2004). Clinically, it has been separated in to non-proliferative and proliferative disease stages. Only the late stages of the retinopathy, especially neovascularization and retinal oedema, have adverse effects on vision, but these disorders seem to be dependent on changes that develop in the earlier stages of the disease. In the ocular lens, the accumulation of polyol induces hyperosmotic swelling and deranges the cell membrane, resulting in the leakage of aminoacids and glutathione to provoke cataract formation (Frank, 2004).

Diabetic Nephropathy: It is now the most common cause of end stage renal failure in the Western World. The main clinical associations that frequently precede overt diabetic nephropathy are hypertension and poor glycemic control (Gilbertson, 2005). It is characterized by the onset of proteinuria and a subsequent decline in glomerular filtration rate and ultimate progression to uraemia, which is fatal if left untreated (Mongensen et al., 1983). Both glucose dependent pathways and other complications of diabetes, and more organ-specific mechanisms that are linked to systematic and intraglomerular hypertension, seem to play important role in the development and progression of this disease (Cooper, 1998).
Due to the limited number of valuable drugs for the treatment of diabetic complications, a number of rational approaches for the discovery of AR inhibitors have been undertaken since the determination of the 3-dimensional structure of the enzyme (Mansour, 2007). Aldose Reductase Inhibition (ARI) represents an attractive strategy for prevention of diabetic complications. The beneficial effect of ARI in preventing or substantially delaying the onset of diabetic complications in experimental models provides strong support to this hypothesis (El-Kabbani et al., 1998). Even, if the therapeutic basis for AR inhibition is valid, efficacy for a given drug might be impossible to establish if the clinical study design does not take into consideration known risk factors for retinopathy such as duration of diabetes, rigor of glycemic control and existence of early changes in retinal vasculature.

Galactosemia

Galactosemia is often confused with diabetes due to the presence of sugar in patient’s urine. However, screening advancements have allowed the exact identity of those sugars to be determined, thereby distinguishing galactosemia (increased galactose level in the blood) from diabetes (increased glucose level in the blood). It is inherited in an autosomal recessive. Heterozygotes are carriers, because they inherit one normal gene and one defective gene. Carriers have been known to show milder symptoms of galactosemia. It is a hereditary disease that results in a defect in, or absence of, galactose-metabolizing enzymes. This inborn error leaves the body unable to metabolize galactose, allowing toxic levels of galactose to build up in blood, cells and tissues. Although treatment for galactosemic infants is a strict galactose-free diet, endogenous production of galactose can cause symptoms such as long-term morbidity, presenile development of cataract, renal failure, cirrhosis and cognitive, neurologic and female reproductive complications (Kinoshita, 1965).
Three distinct types of Galactosemia were identified. They are:

a. GALT (Galactose-1-phosphate uridyltransferase) deficiency.
b. GALK (Galactokinase) deficiency.
c. GALE (UDP-galactose-4′-epimerase) deficiency.

a. **GALT deficiency (Classic galactosemia or Type-1 galactosemia)**

Its symptoms include life threatening illnesses such as jaundice, hepatosplenomegaly (enlarged spleen and liver), hypoglycemia, renal tubular dysfunction, muscle hypotonia (decreased tone and muscle strength), sepsis (presence of harmful bacteria and their toxins in tissues), and cataract among others.

b. **GALK deficiency (Type-2 galactosemia)**

The early onset of cataract is the main clinical manifestation of this galactosemia, most likely due to the high concentration of galactitol found in these persons (Timson and Reece, 2003). The prevalence of cataract among classic galactosemia is markedly less than among galactokinase-deficient patients due to the extremely high levels of galactitol found in the latter (Bosch, 2006).

c. **GALE deficiency (Type 3 galactosemia)**

It is an extremely rare, autosomal recessive disease that appears to be most common among Japanese population. The extreme decrease in GALE activity in the lens of cataract patients is observed and this study suggests an irrefutable connection between Type 3 galactosemia and cataract development (Shin et al., 2000).
AR in Galactose toxicity

AR reduces galactose to its alcohol form, galactitol. Galactitol, however, is not a suitable substrate for the next enzyme in the polyol pathway, polyol dehydrogenase. Therefore galactitol accumulates in body tissues and is excreted in the urine of galactosemic patients. Accumulation of galactitol has been attributed to many of the negative effects of galactosemia, and high concentrations of galactitol have been found in people with classic galactosemia, galactokinase deficiency and epimerase deficiency. In galactosemic patients, the accumulation of galactose becomes the substrate for AR enzyme, which catalyzes the carbohydrate metabolism. In galactosemic cataracts, osmotic swelling of the lens epithelial cells (LEC) occurs. Galactose concentration is fairly high before the enzyme, AR, which will convert significant amounts of the sugar to its galactitol form. The lens is a favourable site for galactose accumulation. The lens phosphorylates galactose at a relatively slow pace in comparison to other tissues. This factor, in combination with low activity of galactose-metabolizing enzymes in galactosemic patients, allows for the accumulation of galactose in the lens. AR is able to dip into this galactose reservoir and synthesize significant amounts of galactitol. It is not a suitable substrate for the enzyme, polyol dehydrogenase, which catalyzes the next step in the carbohydrate metabolic cycle. Thus the sugar alcohol ideally begins to accumulate in the lens (Schoon, 1981). As galactitol concentration increases in the lens, a hypertonic environment is created. Osmosis favours the movement of water into the lens fibers to reduce the high osmolarity. This osmotic movement ultimately results in the swelling of lens fibers until they rupture. Vacuoles appear where a significant amount of osmotic dissolution of fiber has taken place. Interfibrillar clefts filled with precipitated proteins, the manifestation of cataracts is also observed. The progression of galactosemic cataract is generally divided into three stages: initial vacuolar, late vacuolar and nuclear cataract. The formation of a mature, nuclear, cloudy galactosemic cataract typically surfaces 14-15 days after the onset of the galactose diet (Kinoshita, 1965).
AR Inhibitors (ARIs)

AR Inhibitors hinder the AR from synthesizing galactitol in the lens, and thus restricts the osmotic swelling of the lens fiber (Da Settimo et al., 2003). The most commonly available AR inhibitors contained either a cyclicimide groups, such as spirohydantoin group or spirosuccinimide group, an acetic acid moiety.

- Spirohydantoin containing compounds: Sorbinil, Fidarestat and its stereoisomers.
- Acetic acid moiety containing compounds: Tolrestat, Ponalrestat (Statil) and Zopolrestat.

These two groups bind to hydrophilic area of the active side of AR. Another common feature among the various inhibitors is the presence of one or more aromatic groups, which may include phthalazinyl group (Ponalrestat), a naphthyl group (Tolrestat), a benzothiazole group (Zopolrestat), a 2'-thioxo-1,3-thiazolan-4-one group (Epalrestat) and a halogenated benzyl group (Ponalrestat). These aromatic groups bind in the hydrophobic pocket of AR. It was shown that the inhibitors that bind to hydrophobic pocket were better AR inhibitors. A newer class of ARI’s is the phenyl sulphonyl nitromethanes which exhibited potent activity against AR and some of which also showed irreversible inhibition (Mansour, 2007). Many synthetic ARIs have been developed as drug candidates but virtually all have failed although some such as Epalrestat are commercially available in several countries like Japan. Additional ARIs such as Ranirestat, Ponalrestat, Rinalrestat, Risarestat, Ramirestat, Sorbinil and Berberine are currently in clinical trials. ARIs including the acetic acid compounds like Zopolrestat, Tolrestat etc., have not been successful in clinical trials due to adverse pharmacokinetic properties, inadequate efficacy and efficiency, and toxic side effects (Da Settimo et al., 2003).
Plants as source of Aldose Reductase Inhibition

Plants constitute a rich source of bioactive chemicals against AR. Since many of them are largely free from adverse effects and have excellent pharmacological actions, they could lead to the development of new classes of possibly safer therapeutic agents. *Canavalia lineate*, *Cinnamomum cassia*, *Coptis japonica*, *Curcuma longa*, *Fagopyrum esculentum*, *Sorghum bicolor*, and *Vicia tetrasperma* exhibited potent inhibitory activity against rat lens aldose reductase. The methanolic extracts of *Chrysanthemum indicum*, *C. morifolium*, *Prunus mume*, *Myrcia multiflora*, *Centella asiatica* and *Salacia reticulate*, *S. oblonga* and *S. chinensis* exhibited potent inhibitory activity against rat lens aldose reductase (Kim *et al.*, 2001). Other plants with proved ARI activity are, *Withania somnifera*, *Ocimum sanctum*, *Azadirachta indica*, *Curcuma longa* (Halder *et al.*, 2003), *Piper nigrum*, *Momordica charantia*, *Citrus lemon*, *Zingiber officinalis*, *Vitis vinifera*, *Foeniculum vulgare*, *Cuminum cyminum* etc.

Although a number of trials have been conducted over the past 20 years with synthetic compounds used in the treatment of diabetic complications, these trials have yielded minimal results, largely due to the pharmacokinetic problems associated with these aldose reductase inhibitors (ARIs), as well as the length of the trials. At present, only epalrestat is currently available. Thus, an urgent need for new ARIs still exists. Rodents fed on galactose rich diet develop cataract which is morphologically and biochemically similar to cataract found in diabetes (Wolff *et al.*, 1993). The aldose reductase inhibitors reverse the depletion of the plasma ascorbic acid found in experimental diabetes. (Yue *et al.*, 1989) In an ongoing attempt to find powerful, non-toxic and natural ARIs, the fractions of methanol extract (AFSF, MF₂SF and MF₃SF) of bark of *Soymida febrifuga* was evaluated with regard to its potential inhibitory effects aldose reductase.
1.2.8 Liver and liver disorders

The liver is the largest organ in the body, contributing about 1/50 of the total weight of the body. It lies in the upper part of the abdominal cavity. It is described as having four lobes. The two most obvious are the large right lobe and the smaller, wedge shaped, left lobe. The caudate and quadrate lobes are areas on the posterior surface. The lobes of the liver are made of tiny lobules (basic functional unit of liver) just visible to the naked eye. These lobules are hexagonal in outline and are formed by cubical shaped cells, the hepatocytes, arranged in pairs of columns radiating from a central vein. Between two pairs of columns of cells there are sinusoids (blood vessels with incomplete walls) containing a mixture of blood from the tiny branches of the portal vein and hepatic artery (Guyton, 2001).

Their arrangement allows the arterial blood and venous blood (with a high concentration of nutritional materials) to mix and come into close contact with liver cells. Some cells, lining the sinusoids, are hepatic macrophages (Kupffer cells). The posterior surface of the liver is called portal hepatic, where various structures enter and leave the gland. The portal vein enters, carrying deoxygenated blood from the stomach and blood leaving spleen and large intestines. The hepatic artery enters, carrying oxygenated blood. It is a branch from the celiac artery, which is a branch from the abdominal aorta. The right and left hepatic ducts leave, carrying bile from the liver to the gall bladder (Wilson, 1996).

Functions of the liver

Hepatocytes are responsible for many functions that are important in normal functioning of human body, and they can be basically divided into three categories.

i. Regulation and synthesis

ii. Storage

iii. Purification, transformation and clearance
Regulation and Synthesis:

i. Regulation of blood levels of glucose and cholesterol.
ii. Conversion of glucose to glycogen in the presence of insulin, and changing liver glycogen to glucose in presence of glucagon.
iii. Synthesis of most plasma proteins, such as alpha and beta globulin, prothrombin, fibrinogen and very low density lipoproteins.
iv. Synthesis of vitamin A from carotene, the provitamin found in some plants e.g. carrots and green leaves of vegetables.
v. Synthesis of bile salts, which are used in the small intestine for the emulsification and absorption of lipids.
vi. To synthesize cholesterol and use cholesterol to make bile salts.

Purification, transformation and clearance:

i. The liver can detoxify substances such as alcohol, excrete drugs and toxins etc.
ii. It can also chemically alter or excrete thyroid hormones and steroid hormones such as estrogen and aldosterone.

Storage:

i. When blood glucose is high, the liver converts glucose to glycogen and triglycerides for storage.
ii. The liver is a prime storage site for certain vitamins (A, B₁₂, D, E and K) and minerals (iron and copper) which are released from the liver when needed elsewhere in the body (Tortora and Derrickson, 2006).
iii. Deaminates the amino acids so that the amino acids can be used for ATP Production.

iv. The stellate reticulo-endothelial cells (kupffer cells) of the liver are responsible for phagocytosis of aged blood cells, white blood cells and some bacteria (Tortora and Derrickson, 2006).

Liver disorders

Some of the common disorders of the liver include cirrhosis, viral hepatitis, alcoholic liver disease, hemochromatosis, liver cancer, jaundice and drug induced liver damage. Based on their duration of occurrence, they are classified as

i) Acute disorder – occurs over a period less than 3 months
ii) Subacute disorder – lasts for 3 to 6 months
iii) Chronic disorder – lasts for more than 6 months

1) Cirrhosis: It is a widespread and progressive chronic liver condition in which hepatocytes activity is depressed due to excessive amounts of fibrous scar tissue inhibiting blood flow. This blood flow obstruction can cause portal hypertension, which leads to additional complications, including shunting of veins around the liver. Other complications of portal hypertension include swollen veins in the esophagus (varices) and accumulation of fluid in the abdomen (ascites). Other potential complications of cirrhosis include bleeding problems, kidney disorders, osteoporosis, and liver cancer. Any chronic liver disease can eventually lead to cirrhosis, which is believed to be irreversible.

2) Viral hepatitis: The term hepatitis refers to inflammation of the liver. Hepatitis can have several causes, the most common being viruses. Viral hepatitis comes in several forms, the most common being hepatitis B, hepatitis A and hepatitis C. Hepatitis A is infectious hepatitis and is spread by hands, food, water, food contaminated by infected faeces. HVB and HVC are spread by the blood, and can become chronic conditions, which can lead to cirrhosis.
3) **Alcoholic liver disease:** It is of 3 types: alcoholic fatty liver, alcoholic hepatitis, and alcoholic cirrhosis.

**Alcoholic fatty liver:** Fatty liver is the most common, and least harmful. It can occur within days of moderate to heavy drinking. Fat accumulates in the cytoplasm of liver cells, causing the liver to swell, sometimes to large proportions. Fatty liver often has no symptoms, and can disappear as quickly as it appears.

**Alcoholic hepatitis:** It is the inflammation of the liver, and can exist as either acute or chronic conditions. Symptoms can vary greatly, from asymptomatic to severe fever, nausea and abdominal pain. Acute hepatitis can often cause death, and the chronic form often leads to cirrhosis.

**Alcoholic cirrhosis:** It is characterized by regenerative nodules of hepatic tissue completely surrounded by fibrous scar tissue. The scar tissue grows faster than liver cells can regenerate, and the growing network of scar tissue inhibits blood flow.

4) **Hemochromatosis:** It is a condition in which excess amount of iron is present in the body. It is the most common genetic disease. Chronic hemochromatosis can lead to cirrhosis, cancer, impotence, and heart problems. Iron damages the body through its promotion of oxidation, increasing the level of free radicals in the body. Harmful levels of iron can be accumulated in the body simply by eating too much of the wrong foods and supplements.

5) **Liver cancer.** Liver tumors are not always malignant. One type of benign tumor is Hemangioma, which is a non-malignant tumor filled with blood. Malignant tumors fall into two major categories: Metastatic and primary liver tumors. The liver is a frequent target of metastatic cancers, as it the primary filter of venous blood from several organs, such as the colon.
6) **Jaundice:** It occurs due to inability of the hepatocytes to conjugate and excrete bilirubin. Obstruction to the movement of bile through channels by fibrous tissue that has distorted the structural framework of the liver lobules.

7) **Drug induced liver damage:** Many drugs undergo chemical change in the liver before excretion in bile or by other organs. The damage depends on the size of the dose and/or the duration of intake. E.g. include predictable group (Dose related) such as paracetamol, Unpredictable group (individual idiosyncrasy) such as Sulphonamides and Indomethacin.

**A brief account on Herbal Hepatoprotective Agents**

Plant drugs are known to play a vital role in the management of liver disease. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activities (Subramanian et al., 1993). The details of these are summarized in tables – 6,7and 8.

**Table 6. Plants used by the tribal people in the treatment of jaundice**

<table>
<thead>
<tr>
<th>Botanical name</th>
<th>Family</th>
<th>Plant parts used</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acalypha indica</em> L</td>
<td>Euphorbiaceae</td>
<td>2 teaspoons of leaf paste is taken with a cup of curd once in a day for 3 day</td>
</tr>
<tr>
<td><em>Azadirachta indica</em> L</td>
<td>Meliaceae</td>
<td>1 spoonful of roasted flowers is taken along with sugar 3 times a day for about 5 days.</td>
</tr>
<tr>
<td><em>Boerhaavia diffusa</em> L</td>
<td>Nyctaginaceae</td>
<td>2 spoonfuls of plant paste is taken thrice a day for 7 days.</td>
</tr>
<tr>
<td><em>Cassia occidentalis</em> L</td>
<td>Caesalpiniaceae</td>
<td>Leaf juice is mixed with butter milk and 10 spoonful are taken thrice a day for 7 days</td>
</tr>
<tr>
<td><em>Centella asiatica</em> L</td>
<td>Apiaceae</td>
<td>The plant paste is made into pills of 10g each. 2 pills are given 3 times a day for 7 days</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Method</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-----------------</td>
<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Eclipta prostrata</em> L.</td>
<td>Asteraceae</td>
<td>Leaf juice is mixed with curd and 3 spoonfuls of it is given once a day for 7 days.</td>
</tr>
<tr>
<td><em>Erythroxylum monogynum</em> Roxb.</td>
<td>Erythroxylaceae</td>
<td>1-2 spoonfuls of leaf juice is taken twice a day till cure.</td>
</tr>
<tr>
<td><em>Operculina turpenthum</em> L.</td>
<td>Convolvulaceae</td>
<td>2 spoonful of stem bark extract is taken with cold water for 7 days.</td>
</tr>
<tr>
<td><em>Phyllanthus amarus</em></td>
<td>Euphorbiaceae</td>
<td>Whole plant made into paste and mixed with curd 2 spoonful of it is given twice a day for 7 days</td>
</tr>
<tr>
<td><em>Ricinus communis</em> L.</td>
<td>Euphorbiaceae</td>
<td>Tender leaves pound into paste and made into pills. One pill is given once a day on empty stomach for about 7 days</td>
</tr>
<tr>
<td><em>Terminalia pellida Brandis.</em></td>
<td>Combretaceae</td>
<td>Dry fruit kernel powder is mixed with <em>Eclipta prostrata</em> leaf powder and given along with butter milk till cure.</td>
</tr>
<tr>
<td><em>Tribulus terrestris</em> L.</td>
<td>Zygophyllaceae</td>
<td>The plant is made into paste along with equal quantity of whole plant of <em>Amaranthus tricolor</em>, 2 spoonful of paste is mixed with cow milk and given on empty stomach for about a week.</td>
</tr>
<tr>
<td><em>Tridax procumbens</em> L.</td>
<td>Asteraceae</td>
<td>Plant paste with jaggery is taken once in a day for 3-7 days.</td>
</tr>
<tr>
<td><em>Tylophora indica</em></td>
<td>Asclepiadaceae</td>
<td>Root paste is applied on the eyelids for 3 days.</td>
</tr>
<tr>
<td><em>Woodfordia fruticosa</em></td>
<td>Lythraceae</td>
<td>This bark along with the bark of <em>Bauhinia racemosa, Mangifera indica and Oroxylum indicum</em> are taken in equal proportions and made into a paste. 3 times a day for 4-6 days.</td>
</tr>
</tbody>
</table>
Table 7. List of some of the plants reported to have Hepatoprotective and Antihepatotoxic activities

<table>
<thead>
<tr>
<th>Botanical name of the plant</th>
<th>Family</th>
<th>Extract investigated</th>
<th>Phytoconstituents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aegiceras corniculatum</td>
<td>Aegicerataceae</td>
<td>Different extracts of stem (n-hexane, ethyl acetate, methanol)</td>
<td>Flavonoids, Phenolic, Saponins, Sterols (Roome et al., 2008)</td>
</tr>
<tr>
<td>Bacopa monnieri</td>
<td>Schrophulariaceae</td>
<td>Ethanolic extract of aerial parts</td>
<td>Phenols, Polyphenolic, Saponins (Ghosh et al., 2007)</td>
</tr>
<tr>
<td>Cistus laurifolius</td>
<td>Cistaceae</td>
<td>Ethanolic extract of leaves</td>
<td>Flavonoids, diterpenoids, sesquiterpenoids, coumarin and scopoletin (Kupeli et al., 2006).</td>
</tr>
<tr>
<td>Hypericum japonicum</td>
<td>Hypericaceae</td>
<td>Different extracts of whole herb. (Petroleum ether, chloroform, water)</td>
<td>Flavonoids (Wang et al., 2008)</td>
</tr>
<tr>
<td>Hygrophila auriculata</td>
<td>Acanthaceae</td>
<td>Aqueous extract of roots</td>
<td>Polphenolic, steroid, triterpenes, alkaloids proteins, aminoacid (Shanmugasundaram et al., 2006)</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Family</td>
<td>Extract Type</td>
<td>Constituents</td>
</tr>
<tr>
<td>----------------------------</td>
<td>------------</td>
<td>-------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td><em>Nytanthes arboristris</em></td>
<td>Oleaceae</td>
<td>Alcoholic and aqueous extracts leaves</td>
<td>Flavonoids, sterols, tannins, carbohydrates and glycosides (Hukkeri et al., 2006).</td>
</tr>
<tr>
<td><em>Pterocarpus marsupium</em></td>
<td>Papilionaceae</td>
<td>Methanolic extract of stem bark</td>
<td>Marsupin, pterosupin and liquirtigenin (Wongnawa et al., 2006)</td>
</tr>
<tr>
<td><em>Phyllanthus amarus</em></td>
<td>Euphorbiaceae</td>
<td>Ethanol extract of aerial parts</td>
<td>Flavonoids, phenolic, elligatanins (Wongnawa et al., 2006)</td>
</tr>
<tr>
<td><em>Pergularia daemia</em></td>
<td>Asclepiadaceae</td>
<td>Aqueous and ethanol extracts of aerial parts.</td>
<td>Triterpenoids, flavonoids (Suresh Kumar et al., 2006).</td>
</tr>
<tr>
<td><em>Strychnos potatorum</em></td>
<td>Loganiaceae</td>
<td>Aqueous extract of seeds</td>
<td>Triterpenes, steroids, saponins, polphenolic and polysaccharides (Sanmuga priya et al., 2006).</td>
</tr>
<tr>
<td><em>Silybum marianum</em></td>
<td>Asteraceae</td>
<td>Ethanol extract of seeds</td>
<td>Silybin, silychristin, silydianin and isosilybin (Madani, 2008)</td>
</tr>
<tr>
<td><em>Trichilla emetica</em></td>
<td>Meliaceae</td>
<td>Aqueous extracts of roots</td>
<td>Polphenolic, limnoids (Germano et al., 2005)</td>
</tr>
</tbody>
</table>
Table 8. Marketed herbal hepatoprotective formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Company</th>
<th>Ingredients</th>
</tr>
</thead>
</table>
| Livergen Tablets  | Standard                         | *Andrographis paniculata, Cassia angustifolia, Asterchantha longifolia*
|                   |                                  | *Trigonella foenum graecum, Tachyspermum ammi and Apium graveolens.*        |
| Vasuliv Syrup     | Vasu pharma                      | *Phyllanthus niruri, Eclipta alba, Boerhavia diffusa,*                       |
|                   |                                  | *Glycyrrhiza glabra, Terminalia chebula,*                                   |
|                   |                                  | *Picrorrhiza kurrooa,*                                                      |
|                   |                                  | *Tephrosia purpurea,*                                                       |
|                   |                                  | *Cichorium intybus,*                                                        |
|                   |                                  | *Andrographis paniculata.*                                                  |
| Toxi-Gard Capsules| Dalmia Healthcare Limited        | *Azadirachta indica,*                                                       |
|                   |                                  | *Eclipta alba,*                                                             |
|                   |                                  | *Phyllanthus niruri.*                                                       |