Introduction
1.1. INTRODUCTION TO MEDICINAL PLANTS

India has an ancient heritage (Sharma and Deshwal, 2011) of traditional medicine. The materia medica of India provides a great deal of information on the folklore practices and traditional aspects of therapeutically important natural products. Indian traditional medicine is based on various systems including Ayurveda, Siddha, and Unani. The evaluation of these drugs is primarily based on phytochemical, pharmacological, and allied approaches including various instrumental techniques such as chromatography, microscopy, and others. These traditional systems of Indian medicine are each unique but there is a common thread in their fundamental principles and practices. With the emerging worldwide interest in adopting and studying traditional systems and exploiting their potential based on different health care systems, the evaluation of the rich heritage of traditional medicine is essential. The government and the private sector are exploring all of the possibilities for the perfect evaluation of these systems in order to effectively adopt the therapeutic approaches available in original systems of medicine as well as to help in generating data to put these products on the national health program (Mukherjee, 2001).

Botanicals have been used to ameliorate human suffering since antiquity. Even today the active principles derived from botanicals form a major component of therapeutic armamentarium, as about 130 pure chemical entities extracted from about 100 species of higher plants are being used in medicine throughout the world. Most of these have been developed on the basis of information obtained from the traditional medical systems of the west. On the other hand, Ayurveda, the Indian system of medicine remains largely underexplored even through medicinal plants have continued to be an area of major scientific research to discover new therapeutic molecules which can further be used as templates for the development of new therapeutic improved drugs. Many renowned drug houses have launched very active programs of mechanism based natural product discovery research. In one such program, more than 61,000 primary screening assays in 21 different mechanism based assay system yielded less than 0.03 % true leads.

Thus, the mass screening of plants in the search for new drugs is vastly expensive as well as exhaustive with low cost effectiveness. It is well understood that specialized biological screening based on traditional use is more economic and effective. In Ayurvedic texts, there is an exhaustive description of herbs and their
INTRODUCTION

clinical uses which can be further explored using modern scientific methods to establish their therapeutic usefulness and detect the wonder molecules. Ayurveda, thus, has an important role in bioprospecting of further medicine.

Possible because of different therapeutic principles, many difficulties are faced during this process of drug development, which can be adequately overcome by an appropriate correlation of principles for diagnosis and treatment of both the systems of medicine. This in turn depends on an appropriate interpretation of ayurvedic texts in contrast to mere translation in different languages (Arora and Kumar, 2002).

In recent times natural products are becoming an integral part of human health care system, because there is now concern over toxicity and side effects of modern drugs. There is also a realization that natural medicines are safer and allopathic drugs are often ineffective in several ailment. Medicinal plants existed even before human beings made their appearance on the earth. Man's existence on this earth has been made possible only because of the vital role played by plant kingdom in sustaining his life. Since the dawn of civilization, in addition to food crops, man cultivated herbs for his medicinal needs (Dandagi, 2008).

In the last few decades there has been an exponential growth in the field of herbal medicine. It is getting popularized in developing and developed countries owing to its natural origin and lesser side effects. More than 700 mono and polyherbal preparations in the form of decoction, tincture, tablets and capsules from more than 100 plants are in clinical use (Padh and Patel, 2001).

However, there are many limitations regarding safety and efficacy of these preparations. Knowledge about active principles of herbal preparations are not well defined, information on toxicity and adverse effect of these formulations are lacking. Information regarding pharmacokinetics and bioavailability is not available. Packet inserts providing details regarding safety and warning are not required for sale of these, which are available as over the counter preparations. The lay public should know the risk of untested and unregulated remedies. Selection of plant material based on quality, standardization of methods of preparation, enforcement of regulation regarding appropriate labels are measures, which will improve the quality and acceptability of herbal preparations as therapeutic agents. Documentation of research publications in journals and availability of information on website, are other measures to assist research in this field (Kuruvilla, 2002).
INTRODUCTION

The increasing demand for herbal medicines inevitably led to the issue of obtaining and maintaining their quality and purity based on internationally recognized guidelines. WHO has emphasized on the need to ensure the quality control of herbs and herbal formulations by using modern techniques. For pharmaceutical purposes, the quality of medicinal plant material must be as high as that of their medicinal preparations. However, it is impossible to assay a specific chemical entity when the bioactive ingredient is not known. In practice, assay procedures are not carried out even for those medicinal plant materials where there are known active ingredients. Standardization problem arises from the complex composition of drugs, which are used in the form of whole plant, parts of the plant and plant extracts. Standardization of the presumed active compounds of drug in general does not reflect reality. Only in a few cases drug activity depends upon single component. Generally, it is the result of concerted activity of several active compounds as well as of inert accompanying substances. Though these inert accompanying components do not directly affect pathological mechanism, it is reasonable to use the complex mixtures of components provided by a medicinal plant because these inert components might influence bioavailability and excretion of the active component. Further, by inert plant components the stability of the active component might be increased and the rate of side effects also might be minimized. If there are different active compounds present in a plant drug, they might have additive or potentiating effect. The purpose of standardization of traditional remedies is obviously to ensure therapeutic efficacy. The quality assurance of traditional remedies relies upon good manufacturing practices with adequate batch analysis and standardized methods of preparation. Various processes used in manufacture of herbal drugs lack standardized methods.

1.2. HISTORY OF HERBAL MEDICINE

We can certainly assume, however that the healing properties of some plants were discovered by primeval humans fairly early and they learned to use them. By collecting and using medicinal plants, people gained valuable experience good as well as bad and handed down their knowledge to future generations.

One of the first written records concerning curative drugs and narcotic substances was found on a clay tablet in Assyrian cuneiform script dating back to 2,700 BC. The tablet mentions a brown drug, daughter of the poppy, meaning
opium. In ancient Egypt, medicinal science and the use of medicinal substances have an age old tradition. The Egyptian Pharmacopoeia always had a supply of medications of plant and animal, as well as mineral origins. There were 25 types of medicinal plants, as basic nutritional and medicinal plants, onion, garlic, lettuce, lentils, olives and caraway were used (Gupta AK, 2000). The knowledge of Indian physicians is documented by the so-called Bower manuscript found in 1889 in the ruins of MINGSI in central Asia. The document and its author praise the garlic as a panacea claiming it to prolong life to 100 years. In ancient Chinese pharmacology and herbal medicine the most extensive fields of medicine, they contained 8160 prescriptions for the use of various drugs, with instructions on how to use, how to collect and prepare various drugs from medicinal plants.

Ayurveda has well known treaties known as Charak Samhita and Susrut Sanhita, the oldest and very first written document of Ayurveda (900 BC). It describes 341 plants and plant products for use in medicine and more importantly classify these in terms of physiological activity. The traditional medicine used in India known popularly as the Indian system of medicine includes Ayurveda, Siddha, Unani and Naturopathy.

Herbal therapy provides rational means for the treatment of many internal diseases which are considered to be obstinate and incurable in other system of medicine. It lays a great deal of emphasis upon the maintenance of positive health of an individual. It thus aims at both the prevention and cure of diseases. Natural therapy also studies the basic human nature and natural things like hunger, thirst, sleep, sex etc. and provides measures for a disciplined, disease free life and will give a holistic approach to the therapy (Thaibinh, 1998).

1.2.1. WHAT IS A HERBAL MEDICINE?

Herb has various meanings, but in the context of this it refers to "crude drugs of vegetable origin utilized for the treatment of disease states, often a chronic nature, or to attain or maintain a condition of improved health". Herbal preparations called "phytopharmaceuticals" or "phytomedicine" are preparations made from different parts of plants. They come in different formulations and dosage forms including tablet, capsule, elixir, powder, extract, tincture, cream and parenteral preparations. Herbal products in the crude state are also used.
A single isolated active principle derived from plants such as digoxin or reserpine tablets is not considered as herbal medicine. Herbal remedies are not to be confused with homeopathic preparations. Homeopathic medicine, found in the 18th century by the German physician Samuel Hanemann also uses herbs and other natural products, but it is based upon the "Law of similar" and the "Law of dilution".

There is a wealth of non scientific herbal medicine information readily available to the health consumer. Access to scientific literature is crucial to the pharmacist for his or her role as a drug information provider. The pharmacist among all health care practitioners is in the best position to provide information about drug safety and effectiveness. If a herb is used as therapeutic agent it should be considered as a drug (Thaibinh, 1998).

1.2.2. TRADITIONAL MEDICINES

There are several definitions and interpretations of this term, 'traditional medicine'. The most comprehensive is the one where the WHO has defined it as the sum total of all the knowledge and practices, whether explicable or not, used in diagnosis, prevention and elimination of physical, mental or social imbalance relying exclusively on practical experience and observations handed down from generation to generation, whether verbally or in writing (Thaibinh, 1998).

1.2.3. POPULARITY OF HERBAL MEDICINE

The traditional medicine is largely gaining popularity over allopathic medicine because of the following reasons:

1. Rising costs of medical care.
2. As these are from natural origin, they are free from side effects.
3. Goes to root cause and removes it, so that the disease does not occur again.
4. Cure from many obstinate diseases.
5. Easy availability of drugs from natural sources.

1.2.4. NEED AND SCOPE OF HERBAL THERAPY

Today we are more concerned with life style diseases like depression, cancer and heart troubles caused by faulty nutrition and stress. Because these diseases have a mental or emotional component, there is a growing conviction that allopathy is largely
unable to cure them, all it offers is temporary relief from symptoms (Gupta AK, 2000).

Herbal therapy will be one of the best practices to overcome the illness. Traditional Indian practice held that certain drugs should be formulated through the addition of chosen substance that enhances bioavailability of the drug. Recent work, particularly in two Indian modern biology labs, has confirmed the bioavailability enhancer ability of pepper and point to the active component as the molecule piperine. An anti-TB drug rifampicin has to be given at a higher dose than required, in order to compensate for losses on the way to the target site. Formulation of piperine with rifampicin will save the drug and counter effects.

Herbal oriented pharmaceutical companies like Dabur and The Himalaya Drug Company are investing crores of rupees on researching, developing and popularizing OTC remedies. Most of these address modern maladies such as stress, premenstrual syndrome, depression and obesity based on adapted version of ancient Vedic formulas (Thaibinh, 1998).

1.2.5. HERBAL SIDE EFFECTS

Little is known about safety of phytomedicines. There has been an increase in the number of side effects reported in the literature. Many cases, however, could have gone unreported because herbal medicine usually is self-prescribed and often ignored by health practitioners during the patients care. Identifying adverse effects is further hindered because it is not always possible to assess the quality of certain herbal medicinal products (Dandagi et al., 2008).

1.2.6. HERBAL MEDICINE-DRUG INTERACTIONS

The potential risk of a herbal medicine interacting with a prescribed drug is also a concern with the increased use of phytomedicines. Recently, several interactions have drawn the attention of the medical community.

Janetzky and Morreale reported a probable interaction between ginseng, one of the most popular herbs with multiple health claims and warfarin, drug with numerous well recognized drug-drug interactions.

A clinically significant interaction between warfarin and a herbal medicine containing *Salvia miltiorrhiza* roots causing clotting abnormalities. A randomized, cross over study performed on eight healthy subjects reported no significant
pharmacokinetic interactions between Levofloxacin and three selected traditional herbal medicines. No differences were found in Levofloxacin plasma concentration, area under the curve, terminal elimination half-life or renal clearance (Dandagi et al., 2008).

1.2.7. HERBAL MEDICINE ADULTERATION

Standardized herbal preparations are becoming increasingly available. In some parts of the world where no government standards and quality control are enforced, adulteration and contamination pose safety as well as efficacy problems. The presence of adulterants such as arsenic, mercury, lead or prescription drugs in herbal medications can cause significant toxicity. About 23.7% of traditional Chinese medicines (total of 2,609 samples) collected over a year among eight hospitals in Taiwan were spiked with adulterants such as caffeine, acetaminophen, indomethacin, hydrochlorothiazide and other drugs (Dandagi et al., 2008).

1.2.8. HERBAL RESEARCH

The effectiveness, easy availability, low cost and comparatively being devoid of serious toxic effects (time tested) popularized, herbal remedies. Natural products (crude drug extracts and pure compounds) have been derived from higher plants, microbes or animals and these can be of either terrestrial or marine origin. Many of the reputed medicinal plants came under chemical scrutiny leading to the isolation of active principles. Beginning with 1800 A.D., their isolation and characterized extracts became part of pharmacopoeias of several countries. This is where herbal medicine and modern medicine have a common link (Dandagi et al., 2008).

1.2.9. WHY HERBAL REMEDIES?

Herbal medicines are in great demand in developed as well as developing countries for primary healthcare because of their wide biological activities, higher safety margins and lesser costs. They also offer therapeutics for age related disorders like memory loss, osteoporosis, immune disorders, etc. for which no modern medicine is available. Public, academic and government interest in herbal medicines is growing due to the increased incidence of the adverse drug reactions and economic burden of the modern system of medicine (Handa, 1991).
1.2.10. CURRENT SCENARIO IN HERBAL TRADE

India is one of the world’s twelve leading biodiversity centers having over 45,000 different plant species. About 15 - 20 thousand plants are known to have good medicinal properties of which only about 7,000 – 7,500 are being used by traditional medicinal practitioners. The Ayurvedic system of medicine uses around 700, Siddha 600, Unani 700 and modern medicine (Allopathy) about 30 plant species. After information technology, herbal technology will be India’s biggest revenue earner.

Today WHO recommends and encourages traditional herbal remedies in National Health Care Programmes, because these drugs can be made available easily at low cost and are comparatively safe. People’s faith in such remedies is reflected by a hoping annual turnover of Rs. 450 crores of the herbal market. Besides a healthy 11 % annual growth rate, an increasing export potential has attracted several large and medium scale pharmaceutical companies including some Multi-National corporations. However, due to lack of proper quality control measures, the present share of Indian drugs in the world trade is quite insignificant (0.045 %) (Palav and D’mello, 2006).

1.2.11. CONSTRAINTS IN HERBAL TRADE

In India, the herbal drug market is about $ one billion and the export of plant based crude drugs is around $ 80 million. Lack of complete standardization is the most important challenges faced by these formulations. Herbal medicines are prepared from materials of plant origin which are prone to contamination, deterioration and variation in composition. First and foremost task is the selection of the right kind of therapeutically efficacious plant material (Tamizhmani et al., 2003).

1.2.12. NECESSARY STEPS FOR PROMOTING HERBAL DRUGS

Phytochemistry or natural product chemistry research is the backbone of herbal industry and directly/indirectly responsible for both failure and success of herbal drugs. For promoting the use of herbals in modern medicine, phytochemistry should be envisaged for;

- Isolation, purification and characterization of new phytoconstituents.
- Use of newly isolated phytoconstituent as “lead” compound for the synthetic design of analogues with either improved therapeutic activity or reduced toxicity.
Conservation of lead phytoconstituents into medicinally important drugs (Bhanu et al., 2003; Tamizhmani et al., 2003).

1.2.13. ETHNO-PHARMACOLOGICAL APPROACH TO HERBAL DRUGS

The term ethno-pharmacology refers the interdisciplinary scientific observation, description and experimental investigation of indigenous drugs and biological activities. Recent interest in the use of ethno-pharmacological information of plant drugs has greatly increased for several reasons. Scientists showed that of 119 important plant derived drugs used in one or more countries, 88 were regarded as having been discovered as a result of being derived from a plant used in traditional medicine (de Smet, 1989).

1.2.14. PRACTICAL ASPECTS OF HERBAL DRUG DISCOVERY

The following scheme represents a summary of the stages involved in the development of pure drug from a plant source.

- Collection and identification of the plant and deposition of voucher sample in local and major herbaria.
- Literature survey on the plant species selected for studies.
- Extraction with solvent and preparation of non-polar and polar extracts for initial biological testing.
- Evaluation of plant extract against a panel of biological test methods as exemplified by receptor binding, enzyme inhibition and/or cytotoxicity assays.
- Activity guided fractionation on the extract showing activity, by monitoring each chromatographic fraction with bioassay chosen from the panel available to the investigation.
- Structure elucidation of pure active isolate(s) using spectroscopic techniques and chemical methods, if necessary.
- Test each active compound (whether of novel or known chemical structure) in all invitro and invivo biological test methods available, in order to determine potency and selectivity of the drug.
- Perform molecular modeling studies and prepare derivatives of the active compound of interest.
When total synthesis is not practical, carry out large scale re-isolation of interesting active compounds for toxicological, pharmacological and mutation studies.

Clinical trials (phase I – III) (Bhanu et al., 2003).

1.2.15. FUTURE PROSPECTS IN HERBAL MEDICINES

At the moment, scientific research on medicinal plants is continuing most intensely in research institutes, universities and pharmaceutical laboratories as well as in the clinics of many developed countries. This research is oriented mainly in two directions. Firstly the active ingredients of plants that have long been known for their healing properties have been investigated. The second sphere of basic research has led to the discovery of new kinds of medicinal plants and new drugs from the more remote regions of the world where new species with unknown substances still remain to be looked into.

Each and every traditional medicine, drugs of Ayurveda, Unani and Siddha need to be tested and validated scientifically. CSIR, New Delhi, which is already involved in this filed, validated about 350 formulations for different activities. This is a welcome trend since it attempt to marry traditional practice with modern knowledge for the betterment of health. WHO has emphasized the need to ensure the quality control of herbs and herbal formulations by using modern techniques. Several countries have herbal pharmacopoeias and lay down monographs to maintain their quality. Ayurvedic pharmacopoeia of India recommends basic quality parameters for 80 common herbal drugs (Dobriyal and Narayana, 1998b)

1.2.16. PHYTOCHEMISTRY-“AN EMERGING TREND”

Plant based drugs are being increasingly preferred in medical science. The curative parts of a medicinal plant are not simply its woody stem or its leaves, but the number of chemical compounds (phytochemicals) produced and uses for its own growth and development. The therapeutic value and pharmacological action of a drug is due to the presence of certain chemical constituents such as carbohydrates, derivatives of carbohydrates, gums, mucilages, pectins, various forms of glycosides, tannins, phenolic compounds, lipids, fixed and volatile oils, resins, various kinds of alkaloids etc. These phytochemicals are of immense importance to mankind. Phytochemical investigation of plants is an interesting area of research, leading to the
isolation of several new compounds. Though voluminous literature has accumulated on secondary products of plant, very little information is available on their presence and biosynthetic pathways in plants growing in arid zones. Knowledge of chemical constituents of plants is desirable, not only for the discovery of therapeutic agents, but also because such information may be of value in discovering new sources such as tannins, oils, gums, precursors for the synthesis of complex chemical substances etc. In addition, knowledge of the chemical constituents of plants would be valuable in discovering the actual value of folkloric remedies (Naidu et al., 2011).

1.2.17. HERBAL DRUG MARKET

Commercially, plant derived medicine are worth about $14 billion a year in the United States and $ 40 billion worldwide. The average turnover of Indian herbal medicine industry is about 2,300 crore against the pharmaceutical industry which is turned about Rs.14,500 crore with a growth rate of 15 %. However to achieve the goal of major exporter of herbal remedies several steps need to be taken:

a. Systematic study of world market demand and short listing of medicinal herbs with good potential.

b. Systematic cultivation of medicinal herbs on a large scale.

c. Encouragement for agro based phytochemicals and pharmaceutical industries to manufacture value added herbal products.

d. Strict legislation to control quality and purity.

e. Upgradation of cultivation and collection process.

f. Documentation of research work and standardisation for quality (Mukherjee et al., 1998).

The increasing demand for herbal medicines inevitably led to the issue of obtaining and maintaining their quality and purity based on internationally recognized guidelines.

♦ Current Status of Standardization:

WHO has emphasized on the need to ensure the quality control of herbs and herbal formulations by using modern techniques. Several pharmacopoeias like British Herbal Pharmacopoeia (BHP), Japanese Pharmacopoeia (JP), United States Pharmacopoeia (USP), British Herbal Compendium (BHP), German Commission E etc. lay down monographs for herbs to maintain their quality. Ayurvedic Pharmacopoeia of India recommends basic quality parameters for 80 common
Ayurvedic herbal drugs. BHP contains 233 monographs and quality control tests, Chinese Herbal Pharmacopoeia contain 1751 monographs of substances and articles, BHC contains 84 monographs of medicinal plants. German Commission E has 330 monographs for drugs used in German folk medicine.

♦ **Standardization by Marker Compound:**

The best tool developed for standardization is by chromatography. It describes botanical identity and chemical sanctity of herb. One of such technique is marker compound testing and finger print analysis. Secondary metabolites present in herb are considered as marker compounds. Different chromatographic methods are used to analyze the marker compounds in herbs with the help of modern sophisticated tools like HPTLC, HPLC etc (Dobriyal and Narayana, 1998a). List of marker compounds for some herbs are given in table 1.
Table 1: List of marker compounds of some herbs

<table>
<thead>
<tr>
<th>Sr.no.</th>
<th>Name of Herb</th>
<th>Marker Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td><em>Andrographis paniculata</em></td>
<td>Andrographolides</td>
</tr>
<tr>
<td>2.</td>
<td><em>Boerhaavia diffusa</em></td>
<td>Punarnovine</td>
</tr>
<tr>
<td>3.</td>
<td><em>Curcuma longa</em></td>
<td>Curcuminoids</td>
</tr>
<tr>
<td>4.</td>
<td><em>Eugenia caryophylata</em></td>
<td>Eugenol</td>
</tr>
<tr>
<td>5.</td>
<td><em>Glycyrrhiza glabra</em></td>
<td>Glycyrrhizine</td>
</tr>
<tr>
<td>6.</td>
<td><em>Withani somnifera</em></td>
<td>Withanolides</td>
</tr>
<tr>
<td>7.</td>
<td><em>Tinospora cardifolia</em></td>
<td>Giloin</td>
</tr>
<tr>
<td>8.</td>
<td><em>Piper longum</em></td>
<td>Piperine</td>
</tr>
<tr>
<td>9.</td>
<td><em>Ocimum basilicum</em></td>
<td>Eugenol</td>
</tr>
<tr>
<td>10.</td>
<td><em>Zingiber officinale</em></td>
<td>Gingerol</td>
</tr>
</tbody>
</table>
1.3. OVERVIEW ON LIVER DISEASES

Any changes in anatomy or functions of liver are characterized as liver disease. Liver has tremendous capacity to detoxify toxic principle and synthesize useful principles. Therefore damage to the liver inflicted by hepato-toxic agents is of grave consequences. Various types of liver disorders are characterized by cirrhosis, jaundice, tumors, metabolic and degenerative lesions, liver cell necrosis and hepatitis etc. Besides viruses, liver disorders can arise due to xenobiotics, excessive drug therapy and environmental pollution and alcohol intoxication. The management of liver diseases is still a challenge to the modern medicine. The modern allopathic drugs have very little to offer for alleviation of hepatic ailments and some of these drugs adversely affect the liver function. The traditional system of medicine like Ayurveda and Siddha system of medicine, Unani system, Chinese system of medicine, Kampo (Japanese) system of medicine have a major role in the treatment of liver ailments (Kamble et al., 2008).

Treatment of diseases associated with the liver is very vital, and must be done with importance and extensive care. Many herbal remedies for liver diseases are known but only a few of them have been pharmacologically assessed for their efficacy. It is very important to assess natural products for their efficacy in the treatments they are used for. It is especially important to assess remedies for liver diseases due to the liver’s fragility and relation to other vital organs, and its numerous physiological roles in the body (Elsebae and Abu-Zekri, 2008; Kiyosawa et al., 1987; Rosen et al., 1996).

In recent times, due to economic factors, people scramble for available, easily accessible and less costly medication, even with the slightest knowledge of efficacy, and minimum idea of toxicity. Herbal remedies to most people, are natural and thus non-toxic. Toxicity of natural remedies have however been reported, and scientifically proven hepatoprotective plants were found to contain hepatotoxins as well (Bramanti et al., 1978; MacGregor et al., 1989; Oshima, 1995). Thus, work on hepatoprotective herbal remedies remain a challenge (Schuppan et al., 2003).
1.3.1. LIVER

The liver is the heaviest gland of the body. It is inferior to the diaphragm and occupies most of the right hypochondriac part of the epigastric regions of the abdomino pelvic cavity (Tortora and Grabowski, 1996).

The liver is a key organ regulating homeostasis within the body. Functions include protein synthesis, storage and metabolism of fats and carbohydrates, detoxification of drugs and other toxins, excretion of bilirubin and metabolism of hormones. It may be inferred from such broad range of physiological roles that diverse homeostatic mechanisms are affected if liver function is impaired, with potentially serious consequences for the individual concerned (Ward and Daly, 1999).

1.3.2. STRUCTURE OF THE LIVER

The liver is the most important drug-eliminating organ in the body, and is capable of both metabolic transformation and biliary excretion. The liver is a highly specialized and heterogeneous organ, and its structure and attendant heterogeneities must be considered for a more quantitative expression of drug metabolism (metabolite formation) and subsequent metabolism of the metabolites.

The smallest functional unit of the liver is known as the acinus, which consists of a terminal portal venule, hepatic arteriole, a bile duct, lymph vessels and nerves. A zonal relationship exists between the cells constituting the acinus and the blood supply.

The hepatocytes situated close to the portal space are first supplied with fresh blood (rich in oxygen and nutrients), and zonation is contingent upon the oxygen tension at the locale. With a steady input of oxygen entering the organ, a concentration profile in space is created, declining from inlet to outlet. Zone 1 (or periportal region) is closest to the entry of the hepatic artery and portal vein or the portal triad and is having the highest oxygen tension. Zone 3 (perihepatic venous or pericentral region) is near the exit, where the oxygen tension is lowest. An overlapping zone 2 (or midzonal) region exists, where the oxygen tension is intermediate. The different acinar region are specialized in mediating various biochemical/physiological processes. However the shape of profile can be altered as zone 2 cells can be recruited to behave more like zone 1 or 3 cells, depending on flow and oxygen utilization.
The sinusoids are surrounded by single plates of hepatocytes of similar lengths and lined by endothelial cells containing sieve plates with open fenestrae. There is also a freely accessible dissec space (a functional extracellular interstitial space that allows for equilibrative exchange).

The velocities of blood elements flowing through the various sinusoids differ and are displaced as skewed outflow profiles (for dispersion of elements) upon injection of non-eliminated reference indicators (for example, labelled red cell, albumin, sucrose, or water). Functional metabolic and excretory heterogeneities are known to exist, as cells in different zones of the liver are distinct, both morphologically and functionally. Discrete carrier mediated systems present for amino acid or carbohydrate transport have recently been found. This is one type of inbuilt adaptability and protective mechanism of the hepatic system. Any disturbance in this system leads to hepatic disorder. Many drugs/toxins may cause damage to this, which results in the malfunctioning of the liver (Pang et al., 1992; Ross et al., 1995).

1.3.3. ULTRASTRUCTURE OF THE HEPATOCYTE

Hepatic parenchymal cells (hepatocytes) contain a well developed organelle substructure. Mitochondria, which constitute approximately 18 % of the liver cell volume, are the sites of oxidative phosphorylation and energy production. They contain enzymes involved in the citric acid cycle and beta-oxidation of fatty acid.

The rough endoplasmic reticulum is the site of synthesis of many protein, including albumin, coagulation factors, enzymes (e.g. glucose-6-phosphatase), and triglycerides.

The smooth endoplasmic reticulum contains microsomes that are involved with bilirubin conjugation, detoxification (cytochrome P450 dependent isoenzymes), steroid synthesis, cholesterol synthesis, and bile acid synthesis. The enzymes in this system, including gama-glutamyl-transferase, are induced by many drugs and inhibited by others. This is the site of drug metabolism where many important drug interactions occur during the multi-drug therapy. Peroxisomes are found near the smooth endoplasmic reticulum and contain oxidases that utilize molecular oxygen to oxidize a variety of substrates, leading to the production of hydrogen peroxide along with catalase, which decomposes the peroxide. Peroxisomes also catalyze the beta-oxidation of fatty acids and about 5 to 20 % of ethanol metabolism also occurs in peroxisomes. Lysosomes are dense organelles that contain hydrolytic enzymes that
act as scavengers. Deposition of iron, bile pigment and copper occurs in the lysosomes. The Golgi apparatus lies near the canaliculus and is involved with the secretion of various substances, including bile acids and albumin (Tolman and Rej, 1999).

However, during the hepatic injury or damage the homeostasis of enzymatic functions and other cellular organelles is disturbed resulting in the functional and anatomical aberrations of the liver.

1.3.4. PHYSIOLOGICAL AND BIOCHEMICAL ROLES OF THE LIVER

The liver is the largest intra-abdominal solid organ in the human body. It performs various functions essential for the survival of an animal, such as involvement in the processing of fats and proteins from digested foods, the making of proteins essential for blood clotting, the storage of glycogen (which is fuel for the body, made from sugars), the regulation of water distribution between blood and tissues, the processing of some drugs taken into the body (such as acetaminophen and phenytoin), removal of toxins from the body and the production of bile (which contains bile acids and bilirubin) into the gallbladder.

> Management of body fuel

The liver has a unique ability of breaking down glycogen and gluconeogenesis to control blood glucose levels in cells. It removes phosphate from glucose-6-phosphate, forming free glucose to enter the blood. High amount of glucose is stored as glycogen during fed periods. It also synthesizes glucose-6-phosphate from a variety of carbohydrates and from the carbon skeleton of many of the amino acids at a high rate during fasting period.

During fed period, the liver actively synthesizes fat (triglyceride), by converting it to a transportable very low density lipoprotein (VLDL) and releasing into the blood for absorption mainly into adipose tissues as a way of storing excess calories for later use. In fasting period, the triglyceride is broken down into three molecules of free fatty acids and three carbon glycerol molecules. Two molecules of glycerol are converted into one of glucose for use as fuel.

Ketone bodies are important and preferred fuel for some muscle tissues such as heart. The liver solely converts fatty acids to ketone bodies. It degrades free fatty acids to acetyl coA, which in excess, two molecules react to form ketone bodies.
Protein metabolism and nitrogen excretion

About 90% of protein value is re-used in cell systems. This is maintained by synthesizing new molecules of amino acids and breaking down old ones. Amino acid degradation produces carbon structures such as acetyl coA and organic acids involved in the tricarboxylic acid (TCA) cycle. The liver converts excess amino acids into VLDL for storage in fed period, whilst in fasting period; amino acids are converted into proteins for use as fuel, or into other compounds. The liver also uses these amino acids in gluconeogenesis to produce energy.

Nitrogen excretion

Breakdown of amino acids releases the carboxylic acid group and the amino group. It is the sole and unique responsibility of the liver to synthesize urea with excess amino acids in its cells, release to the blood for transportation to the kidneys for concentration and excretion. The alanine cycle makes it possible for the nitrogen to get attached to alanine (synthesized from pyruvate, from glucose) for transportation to the liver and further absorption into liver cells. Nitrogen is released, pyruvate reformed for glucose synthesis and urea synthesized for excretion.

Regulation of water distribution

Water supply to cells is actively done by the circulatory system of heart and blood vessels, and follows the common law of flowing from a high concentrated region to a low concentrated region. At cellular levels, maintenance of osmotic pressure in cells and blood vessels is complemented by the amount of the water-soluble protein, albumin present. Low albumin levels in blood may cause fluid to leak from blood vessels into tissues, resulting in edema. The liver help maintain water balance by solely producing albumin and releasing into blood vessels to control the flow of water, and thus maintaining osmotic pressure.

Detoxification

Toxins are foreign bodies or substances which are harmful to the body, mainly by interfering with cellular processes and causing damage. Exposure to toxins is increasingly becoming unavoidable. They are naturally occurring, from industrial chemicals, and in products we buy, use and even eat on a daily basis such as fertilizers and pesticides used in agricultural production, chemicals used in manufacturing
INTRODUCTION

plastics we use routinely in our homes, packaging materials, food additives, hair products, body creams, etc. Even most pharmaceutical products and drugs meant to manage or treat health conditions are also potentially toxic.

These toxins are eliminated usually by modification of the compounds and excretion by the kidneys. Modification of toxic chemicals and compounds takes place in the smooth endoplasmic reticulum (Jedlickova et al., 1992) where large amounts of different enzymes are found. The main modifying enzyme is a group of enzymes known as the cytochrome-P450 enzymes, which absorbs light of particular frequencies, with a common function of modifying, mainly by oxidizing compounds. Liver cell contains very highly developed SERs, and thus contains large amounts of the body’s cytochrome-P450. Complemented with its rich access to circulatory system makes it effective in the detoxification of foreign bodies. However, chronic exposure to some toxins, or brief exposure to high levels of some organic compounds could cause diseases to the liver (Hamenoo, 2010).

1.3.5. METHODS OF STUDYING HEPATIC DAMAGE

There are various ways by which hepatic damage could be assessed. Methods used are based on principles that govern mechanisms of liver damage and its manifestation. Assessment of biochemical parameters that are hindered in hepatic damage; presence of chemicals and chemical components that are in excess of the normal, evident in hepatic damage; assessment of the body’s ability to metabolize certain chemicals managed by the liver are some methods used in assessing liver damage. Hepatic damage usually manifest in jaundice. Jaundice is the yellowish staining of the skin and sclera (the whites of the eyes) that is caused by high levels of the chemical bilirubin in the blood. When red blood cells get old, they are destroyed. Hemoglobin, the iron-containing chemical in red blood cells that carries oxygen, is released from broken down red blood cells, and the chemical that remains in the blood after the iron is removed is bilirubin. Bilirubin is a waste product removed from the blood by the liver.

After bilirubin has entered liver cells, they are conjugated to other chemicals, primarily glucuronic acid, and the conjugated bilirubin (bilirubin/glucuronate complex) is secreted into bile. This is eliminated through the intestines and the bladder. Jaundice can occur when there is excessive bilirubin production (due to increased and/or rapid breakdown of red blood cells) for removal by the liver, as in
INTRODUCTION

hemolytic anemia; a defect in the liver that inhibits bilirubin removal from the blood; or where there is cholestasis (a defect in the process of bilirubin conjugation, secretion, or flow of bile), such as

- A defect in the liver resulting in cholestasis,
- Blockage of the hepatic ducts and/or the common hepatic duct that decreases the flow of bile from the liver into the gallbladder, as exhibited in cancers and inflammation of the hepatic ducts,
- Blockage of the cystic and/or common bile duct that decreases the flow of bile from the gallbladder and liver, into the intestines, as in cancers, inflammation of the bile ducts and the presence of gallstones.

By evaluating the direct, indirect and total bilirubin levels, the extent of jaundice can be known, and hence the extent of liver damage. In assessing the hepatoprotective ability of a drug in rodent models, determination of the liver enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma glutamic transpeptidase (GGT), and bilirubin levels play a major role. These biochemical parameters reflect damage to the hepatic cells, and are determined clinically with liver function tests (LFTs). ALP and GGT are however generally representative of the hepatobiliary system (the system of liver and the bile ducts). ALP is an enzyme, which is produced, and thus associated with the biliary tract. It is however not specific to the biliary tract because of its presence in bone and placenta. GGT though a more sensitive marker than ALP, may elevate even in minimal levels of liver damage. The enzyme AST also reflects damage to the hepatic cells, but is less specific for liver disease since it is present in minor quantities in red blood cells, cardiac, bone and brain cells, and may be elevated in other conditions such as a myocardial infarct (heart attack). ALT is the enzyme produced within the cells of the liver. The level of ALT is increased in conditions where cells of the liver have been inflamed or undergone cell death. As the cells are damaged, the ALT leaks into the bloodstream leading to a rise in the serum levels. The ratio of direct (conjugated) and/or indirect (unconjugated) bilirubin to total bilirubin is also significantly helpful in determining extent of liver damage.

The extent of damage can also be assessed by pentobarbitone-induced sleep time. Pentobarbitone is a chemical that depresses the central nervous system (CNS), and thus puts the body into an unconscious ‘sleep’ mode when administered. The
INTRODUCTION

Chemical is however metabolized by the liver. Thus, the period it takes the body to recover from unconscious to conscious depicts the period it takes the liver to metabolize the chemical, and thus, the liver’s functional ability.

Homogenous solutions could be made of blood and its light spectrum assessed with a spectrophotometer for various biochemical parameters evident in hepatic damage. Histopathological analysis is another method of assessment useful after lethality due to hepatic injury. This involves excising the liver and making stained slides of liver segments, and observing under an electron microscope for changes in liver structure. Scanning the liver with X-rays is sometimes used to examine the liver for abnormalities. This helps to assess changes in the liver such as inflammation (Hamenoo, 2010).

1.3.6. INDUCTION OF HEPATIC DAMAGE

Liver injury is caused by various factors, such as viral infections, poisoning from alcohol, chronic bacterial infections and drugs, over periods of exposure. Experimentally, hepatic damage is induced using quick effective and reliable methods that give reproducible effects, with minimum lethality expectation (Jang et al., 2008). This is usually attained experimentally by chemical means.

- **Acetaminophen induced hepatotoxicity**

  Acetaminophen (paracetamol) was metabolically activated by cytochrome P450 enzymes to a reactive metabolite that depleted glutathione (GSH) and covalently bound to protein. It was shown that repletion of GSH prevented the toxicity. This finding led to the development of the currently used antidote N-acetylcysteine. The reactive metabolite was subsequently identified to be N-acetyl-p-benzoquinone imine (NAPQI). Although covalent binding has been shown to be an excellent correlate of toxicity, a number of other events have been shown to occur and are likely important in the initiation and repair of toxicity. Recent data have shown that nitratet tyrosine residues as well as acetaminophen adducts occur in the necrotic cells following toxic doses of acetaminophen. Nitrotyrosine was postulated to be mediated by peroxynitrite, a reactive nitrogen species formed by the very rapid reaction of superoxide and nitric oxide (NO). Peroxynitrite is normally detoxified by GSH, which is depleted in acetaminophen toxicity. NO synthesis (serum nitrate plus nitrite) was dramatically increased following acetaminophen. In inducible nitric oxide synthase
INTRODUCTION

(iNOS) knockout mice, acetaminophen did not increase NO synthesis or tyrosine nitration; however, histological evidence indicated no difference in toxicity. Acetaminophen did not cause hepatic lipid peroxidation in wild-type mice but did cause lipid peroxidation in iNOS knockout mice. These data suggest that NO may play a role in controlling lipid peroxidation and that reactive nitrogen/oxygen species may be important in toxicity. The source of the superoxide has not been identified, but our recent finding that NADPH oxidase knockout mice were equally sensitive to acetaminophen and had equal nitration of tyrosine suggests that the superoxide is not from the activation of Kupffer cells. It was postulated that NAPQI-mediated mitochondrial injury may be the source of the superoxide. In addition, the significance of cytokines and chemokines in the development of toxicity and repair processes has been demonstrated by several recent studies. IL-1β is increased early in acetaminophen toxicity and may be important in iNOS induction. Other cytokines, such as IL-10, macrophage inhibitory protein-2 (MIP-2), and monocyte chemotactic protein-1 (MCP-1), appear to be involved in hepatocyte repair and the regulation of proinflammatory cytokines (James et al., 2003).

Figure 1: Schematic representation depicting the role of metabolism in acetaminophen toxicity
Carbon tetrachloride induced hepatotoxicity

Carbon tetrachloride is a known hepatotoxic agent used to induce liver injury due to its toxicity to hepatocytes that produces effects histopathologically similar to that of viral hepatitis. Its mechanism of action has been however obscure. Recent investigation conducted found mitochondria to be the main cell component attacked by the drug (Christie and Judah, 1954). In this process, the tricarboxylic acid cycle is disorganized by the inhibition of the oxidation of citrate, malate, pyruvate and glutamate, 10 to 15 hours after administration of the drug. This is resulted in a disorganization of the enzyme systems. The mitochondria affected were found to lose pyridine nucleotides, and allow penetration of Co I, at abnormally fast rates, than in normal mitochondria.

1.3.7. OTHER CAUSES OF DISEASES OF LIVER

The liver injury may take several forms and involve the hepatocytes, vascular cells or bile ducts. The most important disease are

- **Biliary obstruction:**
  Bile flow obstruction results in jaundice. Lesions in the main extra hepatic bile duct, such as carcinoma, impacted bile stones, or sclerosing cholangitis typically cause obstructive jaundice. Prolonged bile duct obstruction may cause secondary biliary cirrhosis (Omer, 2005)

- **Metabolic disorders:**
  Metabolic disorder of the liver may be hereditary (genetic) or acquired. Representative hereditary hyperbolic rubinemias and disturbances involving the intermediate metabolism of lipids, carbohydrates, proteins and heavy metals are few examples of metabolic disorders of liver (Omer, 2005).

- **Congenital metabolic disorder:**
  Congenital hyperbilirubinemia occurs in several forms. The best-known congenital jaundice syndromes are Gilbert syndrome, Rotor syndrome, and Dubin – Johnson syndrome. Genetic enzyme deficiencies such as alpha–1–antitrypsin deficiency may also result in liver injury, which ultimately lead to cirrhosis (Omer, 2005).
- **Acquired metabolic disorders:**
  Metabolic disorder can be induced in liver cells by a variety of ingested substances such as toxins, drugs, foods and beverages. Alcohol produces three types of liver disease such as hepatomegaly, alcoholic hepatitis and cirrhosis. Several drug for example methyldopa, nitrofurantoin, isoniazid, ketoconazole and acetaminophen, etc., can induce hepatitis (Omer, 2005).

- **Viral hepatitis:**
  Acute viral hepatitis is a systemic infection manifested primarily by an acute attack on the hepatocytes. Five hepatotropic viruses have been identified (HAV, HBV, HCV, HDV, HEV). Hepatitis A (HAV) causes acute, self-limited disease that is transmitted orally. Hepatitis B (HBV) and Hepatitis C Viruses (HCV) are transmitted by exchange of body fluids such as through blood transfusion or sexual contacts. Hepatitis D viruses (HDV) are a viroid that causes inflammation only in concrete with HBV. Hepatitis E virus is transmitted by enteric route and cause self-limited diseases.

  Chronic hepatitis is an uncommon, but important, complication of HBV and combined HBV-HDV infection. The liver injury results from an inflammatory immune attack against hepatocytes (Omer, 2005).

- **Cirrhosis:**
  Cirrhosis is a chronic liver disease characterized by wide spread fibrosis and regenerative nodules, which diffusely replace the normal liver parenchyma. The major causes of cirrhosis are alcoholism and viral hepatitis (HBV, HCV and HDV) (Omer, 2005).

- **Liver tumor:**
  Primarily liver tumors may originate from liver cells, from bile ductules and less often from kupffer cells and connective tissue cells of hepatic capsule and portal tracts. Hepatocellular carcinoma (malignant hepatonia) is the most common primary malignant liver tumor. Cholangio cellular carcinoma is a malignant tumor of bile ducts (Omer, 2005).
• **Necrosis**:
  
  Hepatic necrosis may be zonal, massive or diffusive. Zonal necrosis may be in the central, peripheral or midzone of the lobule, depending on the agent. In general, the necrosis that is produced by intrinsic (predictable) hepatotoxins is zonal; while that produced by an idiosyncratic reaction to a drug is usually diffusive or massive. Centrizonal necrosis is the characteristic lesion produced by carbontetrachloride, chloroform, iodoform, bromobenzene and other series of acetaminophen (Omer, 2005).

• **Degeneration**:
  
  Agents that lead to necrosis also lead to degeneration of hepatocytes that is evident from the fact that, prior to the development of necrosis or at its periphery, hepatocytes show sub-necrotic damage that includes hydropic degeneration or balloning and eosinophilic degeneration, the latter leading to the free sinusoidal, acidophilic bodies (Omer, 2005).

• **Steatosis**:
  
  A large number of agents can produce a fatty liver. Two main types of fatty change occur, some agents (viz; tetracycline and a number of experimental toxins) produce microvesicular steatosis. In this form of fatty liver, the hepatocytes are filled with tiny fat droplets that do not displace the nucleus. Other agents (alcohol, methotrexate) lead to macrovascular steatosis. This is the more familiar form in which most of the fatty cells contains a large droplet of fat which displaces the nucleus to the periphery (Omer, 2005).

• **Cholestatic Injury**:
  
  Liver injury induced by viri, chemicals and drugs is a well-recognized toxicological problem. The hepatotoxicity possibly results from a toxic intermediary that binds covalently to hepatocytes and causes a centrilobular hepatic necrosis. Alternate explanations of necrosis are lipid peroxidation and oxidation of thiol group. One of the major causes of carbon tetrachloride (CCl₄) induced hepatopathy is lipid peroxidation by its free radical derivative, CCl₃ (Castro et al., 1974).
Inhibition of generation or scavenging (antioxidant activity) of free radicals plays a crucial role in providing protection against such hepatic damage (Chang et al., 1995; Lin et al., 1998).

- **Lipid peroxidation:**
  Lipids are major constituents of membranes. In addition to the fundamental role of providing compartmentation, the cell membrane lipids are involved in the responses of cells to a number of external stimuli like hormones, growth factors and neurotransmitters (Bindu and Babu, 2001).

  Lipid peroxidation is oxidative destruction of polyunsaturated fatty acids (PUFA) in the cell membrane. Oxygen radicals and active forms of oxygen are generated in many redox processes. The cells have built in antioxidant systems to check this deleterious process. These include enzymes like catalase, superoxide dismutase, glutathione reductase etc. as well as non-enzymatic molecules like glutathione, vit E, carotene etc. (Ames et al., 1993). Overproduction of free radicals, associated with A, C and E vitaminosis and a reduced level of the above mentioned enzymes, seems to be the main factor which lead to the so called oxidative stress (Nuutila et al., 2003).

  Therefore, superoxide dismutase, glutathione reductase and lipid peroxidation levels are treated as indicators of oxidative stress on the liver and their estimation is considered as very important manifestations of disturbed liver functions.

- **Biochemical and functional manifestations of injury:**
  In toxicity much attention is devoted to the effect of drugs or chemicals on liver function. Since the liver is an organ with such diverse functional activity, no single parameter can be selected as the indicator of “liver function”. The development of the so-called liver function test has largely followed the development of new knowledge about the biochemistry of the liver. Liver may play a major role in defining the biological activity of the substance in question; the test itself indirectly becomes a measure of hepatic function. In the present study paracetamol induced hepatically injured models are being adapted for screening the hepatoprotective activity.

  Reports showed that, levels of several biologically important enzymes were elevated in paracetamol induced hepatic injury. Enzymes in the serum that are altered during hepatic injury are glutamate oxaloacetate transaminase (GOT), glutamate
pyruvate transaminase (GPT), ornithine carbamoyl transferase (OCT), maleic dehydrogenase (MDH), aldolase (ALD), fructose-1-phosphate aldolase (F-1-P-AD), glutathione reductase (GR), lactic dehydrogenase (LDH), glutamate dehydrogenase (GDH), cathepsin-B (CLP-B), sucrose-6-phosphatase (G-6-Phos), ribonuclease (Riba), acid phosphatase (ACP), alkaline phosphatase (ALP) (Omer, 2005).

- **Transaminases:**

  Enzymes called ‘transaminases’ catalyse the transfer of amino group of an amino acid to an alfa keto acid to form a new amino acid and a new keto acid. Alanine, aspartic acid etc. are formed in this way.

  A proportionate relationship between the degree of elevation in serum enzyme activity and the degree of liver necrosis. SGOT (Serum Glutamate Oxaloacetate Transaminase) and SGPT (Serum Glutamate Pyruvate Transaminase) levels increase in blood plasma on account of their release from the liver tissue, which is damaged.

  In most clinical circumstances this is demonstrated by elevated levels of the transaminases which may increase from 10 to 100 fold than the normal SGOT, SGPT activity. The extent of elevation in the levels of these enzymes depends upon the type and severity of damage caused by the hepatotoxic agents (Omer, 2005).

- **Bilirubin levels:**

  One of the normal functions of the liver is to excrete the breakdown product of hemoglobin-bilirubin into bile. Therefore, the measurement of the liver ability to remove bilirubin from the blood and to excrete bilirubin into the bile can be used as a liver function test. In this situation, this abnormal state can only be detected by estimating the plasma bilirubin levels.

  Elevation of bilirubin level of the serum accompanies, severe parenchymal injury, but it is a relatively insensitive measure of chemical hepatic injury. Substances like CCl₄, do cause sufficient parenchymal injury to cause a large increase in SGPT activity but does not affect bilirubin level very greatly. It appears, therefore, bilirubin clearance is not a sensitive index of hepatic dysfunction, unless a marked defect has occurred on the biliary excretory phase of this organ (Tietz and Aldrich, 1987).
**Cholesterol values:**

A rise in the lipid content of liver much beyond the usual 5% is an abnormal condition and is described as fatty liver. Toxic factors are one of the reasons for the same. CCl₄ impairs protein synthesis and also the conjugation of protein with lipid. The secretion of lipoprotein by the liver cell is thus impaired (Robert et al., 2000).

**Hepatotoxicity:**

Although drugs are usually metabolized without injury to the liver, many fatal and near fatal drug reactions occur each year. Factors promoting the accumulation of hepatocyte toxins include genetic alterations in enzymes that allow the formation of the harmful metabolites, competition by other drugs and depletion of the substrates required to detoxify the metabolites.

A few compounds produce metabolites that cause liver injury in a uniform, dose dependent fashion. Injury to hepatocytes results in either directly from the disruption of intracellular functions or membrane integrity or indirectly from immune-mediated membrane damage (Gennaro, 2000).

• **Types of hepatotoxic agents:**

The central role of the liver in drugs/toxins metabolism predisposes it to toxic injury, not only by the bioaccumulation of drugs/toxin in the liver but also because metabolism may go away, leading to the formation of electrophilic toxic metabolites.

These toxic metabolites, however, can be detoxified by a third phase in which they react with glutathione. Glutathione is available in limited supply and can be depleted by alcohol ingestion or fasting, both of which predispose to drug/toxin-induced injury (Zimmerman and Ishak, 1994). The drugs/chemicals/toxins that are causing hepatic injury are classified in table 2 and 3.
### Table 2: Classification of hepatotoxic substances

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INORGANIC AGENTS</strong></td>
<td>Metals and metalloids: antimony, arsenic, beryllium, bismuth, boron, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury, gold, phosphorous, selenium, tellurium, thallium, zinc, hydrazine derivative, iodides.</td>
</tr>
<tr>
<td><strong>ORGANIC AGENTS</strong></td>
<td></td>
</tr>
<tr>
<td>Natural: Plant toxins</td>
<td>Albitocin, cycasin, nutmeg, tannic acid, icterogenin, pyrrolidizines, saferole, indospicine</td>
</tr>
<tr>
<td>Mycotoxins</td>
<td>Aflatoxins, cyclochlorotine, ethanol, luteoskyrin, griseofulvin, sporidesmin, tetracycline, and other antibiotics.</td>
</tr>
<tr>
<td>Synthetic Non-medicinal agents</td>
<td>Haloalkanes and haloolefins, nitroalkanes, chloroaromatic compounds, nitroaromatic compound, organic amines, azo compounds. phenol and derivatives, various other organic compounds</td>
</tr>
<tr>
<td><strong>MEDICINAL AGENTS</strong></td>
<td></td>
</tr>
<tr>
<td>1) Neuro psychotropics</td>
<td>Hydrazine, tranylcypromine anticonvulsants, antidepressants.</td>
</tr>
<tr>
<td>2) Anti-inflammatory and anti-muscle spasm agents</td>
<td>Cinchopen, cholchicine, ibuprofen, salicylates, indomethacin.</td>
</tr>
<tr>
<td>3) Hormonal derivatives and other drugs used in endocrine disease</td>
<td>Acetohexamide, azepinamide, carbutamide, tolbutamide.</td>
</tr>
<tr>
<td>4) Antimicrobials</td>
<td>Clindamycin, novobiocin, penicillin, tetracycline, sulfonamide, amodiaquine, isoniazid, rifampin.</td>
</tr>
<tr>
<td>5) Antineoplastic</td>
<td>L-asparaginase, azacytidine, methotrexate, 6-mercaptopurine, chlorambucil, clavacin.</td>
</tr>
</tbody>
</table>
Table 3: Clinically important hepatotoxins and their mechanism in causing hepatotoxicity (Curtis, 2001; Zimmerman and Ishak, 1994).

<table>
<thead>
<tr>
<th>Category of agents</th>
<th>Mechanism of action</th>
<th>Histologic lesion</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Direct</td>
<td>Direct physiochemical destruction by peroxidation of hepatocytes.</td>
<td>Necrosis and/or steatosis</td>
<td>CCl₄, phosphorus</td>
</tr>
<tr>
<td>Indirect cytotoxic</td>
<td>Interference with hepatocellular metabolic pathways</td>
<td>Steatosis or necrosis</td>
<td>Ethionine, ethyl alcohol</td>
</tr>
<tr>
<td>Cholestatic</td>
<td>Interference with bile excretory pathways</td>
<td>Cholestasis destruction</td>
<td>Methylene dianiline, anabolic and contraceptive steroids</td>
</tr>
<tr>
<td>Host Idiosyncracy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypersensitivity</td>
<td>Drug allergy</td>
<td>Necrosis or cholestasis</td>
<td>Chlorpromazine, phenytion, sulfonamides</td>
</tr>
<tr>
<td>Metabolic</td>
<td>Production of hepatotoxic metabolites</td>
<td>Necrosis or cholestasis</td>
<td>Isoniazid, valproic acid</td>
</tr>
</tbody>
</table>
1.3.8. CURRENT MANAGEMENT OF LIVER DISEASES

Management of liver diseases involves the management of jaundice (since bilirubin is a waste product which can be toxic to the system) and management of the root cause of the jaundice, then the cause of the liver disease. Hepatoprotectants used for the management of hepatitis varies from orthodox through homoeopathy to botanic medical therapies.

Silymarin, multivitamins, methionine, ursodeoxycholic acid and liver hydrolysate have also been used to manage liver diseases (Chamulitrat et al., 2009). Nitric oxide, a cytoprotectant, inhibits cell destruction by modulating heat shock proteins, S-nitrosylating caspases at their catalytic site cysteine residue, triggering the cGMP pathway, and preventing mitochondrial dysfunction (Wang et al., 2010). Presently, there hasn’t been found any synthetic hepatic damage remedy safe enough to give therapy effectively, yet without severe side effects.

In addition, no hepatic damage remedy has been found to give complete and perpetual cure to hepatic injuries, without relapses or resurfacing of the disease. Medicinal products used are found to give only symptomatic relief to patient with hepatic disorder without managing the fundamental cause to the symptoms (ji Ram and Goel, 1999).

In Homoeopathy, liver injury, depending on its symptoms, cause and extent of damage, is managed by a variety of drugs, some of which are bryonia, mercurius, podophyllum, chelidonium and digitalis.

Natural hepatoprotectants are used, and though not well investigated, are claimed to be effective in controlling hepatic disorders while limiting the side effects of the drug. Botanically, many hepatoprotectants have been reported. *Glycosmis arborea* extract was able to overcome the toxic effects of hepatotoxic agents in terms of lowering the levels of serum GPT, alkaline phosphatase necrosis of liver produced by carbon tetrachloride was reversed by the extract (Gomes et al., 2003).
1.4. EXCIPIENTS PROFILE

1.4.1. CELLULOSE, MICROCRYSTALLINE

✦ Nonproprietary names

BP: Microcrystalline Cellulose
JP: Microcrystalline Cellulose
PhEur: Cellulose, Microcrystalline
USP-NF: Microcrystalline Cellulose

✦ Synonyms

Avicel PH; Cellets; Celex; cellulose gel; hellulosum microcristallinum; Celphere; Ceolus KG; crystalline cellulose; E460; Emcocel; Ethispheres; Fibrocel; MCC Sanaq; Pharmacel; Tabulose; Vivapur.

✦ Chemical name and CAS registry number

Cellulose [9004-34-6]

✦ Empirical formula and molecular weight

\[(\text{C}_6\text{H}_{10}\text{O}_5)_n \approx 36\,000\]

where \( n \approx 220.\)

✦ Structural formula

![Cellulose Structural Formula](image)

✦ Functional category

Adsorbent; suspending agent; tablet and capsule diluent; tablet disintegrant

✦ Description

Microcrystalline cellulose is purified, partially depolymerized cellulose that occurs as a white, odorless, tasteless, crystalline powder composed of porous particles. It is commercially available in different particle sizes and moisture grades that have different properties and applications.
Applications in pharmaceutical formulation or technology

Microcrystalline cellulose is widely used in pharmaceuticals, primarily as a binder/diluent in oral tablet and capsule formulations where it is used in both wet granulation and direct compression processes. In addition to its use as a binder/diluent, microcrystalline cellulose also has some lubricant and disintegrant properties that make it useful in tableting (Rowe et al., 2009).

1.4.2. STARCH

Nonproprietary names

BP: Maize starch, Potato starch, Rice Starch, Tapioca Starch, Wheat Starch
JP: Corn Starch, Potato Starch, Rice Starch, Wheat Starch
PhEur: Maize Starch, Pea Starch, Potato Starch, Rice Starch, Wheat Starch
USP-NF: Corn Starch, Potato Starch, Tapioca Starch, Wheat Starch

Synonyms

Amido; amidon; amilo; amyllum; PharmGel; Eurylon; fecule; Hylon; maydis amyllum; Melojel; Meriten; oryzae amyllum; Pearl; Perfectamyl; pisi amyllum; Pure-Dent; Purity 21; Purity 826; solani amyllum; tritici amyllum; UniPure

Chemical name and CAS registry number

Starch [9005-25-8]

Empirical formula and molecular weight

$(C_{6}H_{10}O_{5})_{n}$ where $n \approx 300–1000$.

Structural formula
INTRODUCTION

- **Functional category**
  Tablet and capsule diluent; tablet and capsule disintegrant; tablet binder; thickening agent.

- **Description**
  Starch occurs as an odorless and tasteless, fine, white to off-white powder. It consists of very small spherical or ovoid granules or grains whose size and shape are characteristic for each botanical variety.

- **Applications in pharmaceutical formulation or technology**
  Starch is a versatile excipient used primarily in oral solid-dosage formulations where it is utilized as a binder, diluent, and disintegrant. As a diluent, starch is used for the preparation of standardized triturates of colorants, potent drugs, and herbal extracts, facilitating subsequent mixing or blending processes in manufacturing operations. Starch is also used in dry filled capsule formulations for volume adjustment of the fill matrix, and to improve powder flow, especially when using dried starches. Starch quantities of 3 - 10 % w/w can act as an antiadherent and lubricant in tableting and capsule filling.

  In tablet formulations, freshly prepared starch paste is used at a concentration of 3 – 20 % w/w (usually 5 – 10 %, depending on the starch type) as a binder for wet granulation. The required binder ratio should be determined by optimization studies, using parameters such as tablet friability and hardness, disintegration time, and drug dissolution rate. Starch is one of the most commonly used tablet disintegrants at concentrations of 3 – 25 % w/w; a typical concentration is 15 %. When using starch, a prior granulation step is required in most cases to avoid problems with insufficient flow and segregation. A starch–lactose compound has been introduced enabling the use of granular starch in direct compression, improving the tableting process and the disintegration time of the tablets.

  However, starch that is not pregelatinized does not compress well and tends to increase tablet friability and capping if used in high concentrations; Starch, particularly the fine powders of rice and wheat starch, is also used in topical preparations for its absorbency of liquids. Starch paste is used in ointment formulations, usually in the presence of higher ratios of glycerin. Starch has been investigated as an excipient in novel drug delivery systems for nasal, and other site-specific delivery systems. The retro gradation of starch can be used to modify the surface properties of drug particles. Starches are useful carriers for amorphous
drug preparations, such as pellets with immediate or delayed drug release obtained, for example, by melt extrusion, and they can improve the bioavailability of poorly soluble drugs. Starch, particularly rice starch, has also been used in the treatment of children’s diarrheal diseases.

Specific starch varieties with high amylose content (resistant starches) are used as insoluble fiber in clinical nutrition, and also for colon-targeting applications. Due to their very high gelatinization temperature, these starches are used in extrusion/spheronization processes (Rowe et al., 2009).

1.4.3. AEROSIL

▪ **Nonproprietary names**
  
  BP: Colloidal Anhydrous Silica  
  JP: Light Anhydrous Silicic Acid  
  PhEur: Silica, Colloidal Anhydrous  
  USP-NF: Colloidal Silicon Dioxide

▪ **Synonyms**
  
  Aerosil; Cab-O-Sil; Cab-O-Sil M-5P; colloidal silica; fumed silica; fumed silicon dioxide; hochdisperses silicum dioxid; SAS; silica colloidal is anhydrica; silica sol; silicic anhydride; silicon dioxide colloidal; silicon dioxide fumed; synthetic amorphous silica; Wacker HDK.

▪ **Chemical name and CAS registry number**
  
  Silica [7631-86-9]

▪ **Empirical formula and molecular weight**
  
  $\text{SiO}_2$ 60.08

▪ **Structural formula**
  
  $\text{SiO}_2$

▪ **Functional category**
  
  Adsorbent; anticaking agent; emulsion stabilizer; glidant; suspending agent; tablet disintegrant; thermal stabilizer; viscosity increasing agent

▪ **Description**
  
  Colloidal silicon dioxide is submicroscopic fumed silica with a particle size of about 15 nm. It is a light, loose, bluish-white colored, odorless, tasteless, amorphous powder.
Applications in Pharmaceutical Formulation or Technology

Colloidal silicon dioxide is widely used in pharmaceuticals, cosmetics, and food products. Its small particle size and large specific surface area give it desirable flow characteristics that are exploited to improve the flow properties of dry powders in a number of processes such as tableting and capsule filling.

Colloidal silicon dioxide is also used to stabilize emulsions and as a thixotropic thickening and suspending agent in gels and semisolid preparations. With other ingredients of similar refractive index, transparent gels may be formed. The degree of viscosity increase depends on the polarity of the liquid (polar liquids generally require a greater concentration of colloidal silicon dioxide than non polar liquids). Viscosity is largely independent of temperature. However, changes to the pH of a system may affect the viscosity. In aerosols, other than those for inhalation, colloidal silicon dioxide is used to promote particulate suspension, eliminate hard settling, and minimize the clogging of spray nozzles.

Colloidal silicon dioxide is also used as a tablet disintegrant and as an adsorbent dispersing agent for liquids in powders. Colloidal silicon dioxide is frequently added to suppository formulations containing lipophilic excipients to increase viscosity, prevent sedimentation during molding, and decrease the release rate. Colloidal silicon dioxide is also used as an adsorbent during the preparation of wax microspheres; as a thickening agent for topical preparations; and has been used to aid the freeze drying of nanocapsules and nanosphere suspensions (Rowe et al., 2009).

1.4.4. TALC

Nonproprietary names

BP: Purified Talc
JP: Talc
PhEur: Talc
USP: Talc

Synonyms

Altalc; E553b; hydrous magnesium calcium silicate; hydrous magnesium silicate; Imperial; Luzenac Pharma; magnesium hydro-gen metasilicate; Magsil Osmanthus; Magsil Star; powdered talc; purified French chalk; Purtalc ; soapstone; steatite; Superiore ; talcum.
• **Chemical name and CAS registry number**
  
  Talc [14807-96-6]

• **Empirical formula and molecular weight**
  
  Talc is a purified, hydrated, magnesium silicate, approximating to the formula \( \text{Mg}_6(\text{Si}_2\text{O}_5)_4(\text{OH})_4 \). It may contain small, variable amounts of aluminum silicate and iron.

• **Structural formula**
  
  \( \text{Mg}_6(\text{Si}_2\text{O}_5)_4(\text{OH})_4 \)

• **Functional Category**
  
  Anticaking agent; glidant; tablet and capsule diluent; tablet and capsule lubricant

• **Description**
  
  Talc is a very fine, white to grayish-white, odorless, impalpable, unctuous, crystalline powder. It adheres readily to the skin and is soft to the touch and free from grittiness.

• **Applications in pharmaceutical formulation or technology**
  
  Talc was once widely used in oral solid dosage formulations as a lubricant and diluents. Although today it is less commonly used. However, it is widely used as a dissolution retardant in the development of controlled release products. Talc is also used as a lubricant in tablet formulations; in a novel powder coating for extended release pellets; and as an adsorbant.

  In topical preparations, talc is used as a dusting powder, although it should not be used to dust surgical gloves. Talc is a natural material; it may therefore frequently contain microorganisms and should be sterilized when used as a dusting powder. Talc is additionally used to clarify liquids and is also used in cosmetics and food products, mainly for its lubricant properties (Rowe et al., 2009).
1.4.5. MAGNESIUM STEARATE

- **Nonproprietary names**
  - BP: Magnesium stearate
  - JP: Magnesium stearate
  - PhEur: Magnesium stearate
  - USP-NF: Magnesium stearate

- **Synonyms**
  - Dibasic magnesium stearate; magnesium distearate; magnesia stearas; magnesium octadecanoate; octadecanoic acid, magnesium salt; stearic acid, magnesium salt; Synpro 90.

- **Chemical name and CAS registry number**
  - Octadecanoic acid magnesium salt [557-04-0]

- **Empirical formula and molecular weight**
  - C_{36}H_{70}MgO_4, 591.24

- **Structural formula**
  - \([\text{CH}_3\text{(CH}_2\text{)}_{16}\text{COO}]_2\text{Mg}\)

- **Functional category**
  - Tablet and capsule lubricant

- **Description**
  - Magnesium stearate is a very fine, light white, precipitated or milled, impalpable powder of low bulk density, having a faint odor of stearic acid and a characteristic taste. The powder is greasy to the touch and readily adheres to the skin.

- **Applications in pharmaceutical formulation or technology**
  - Magnesium stearate is widely used in cosmetics, foods, and pharmaceutical formulations. It is primarily used as a lubricant in capsule and tablet manufacture at concentrations between 0.25 % and 5.0 % w/w. It is also used in barrier creams (Rowe et al., 2009).
1.4.6. CROSPovidONE

- **Nonproprietary names**
  - BP: Crospovidone
  - PhEur: Crospovidone
  - USP-NF: Crospovidone

- **Synonyms**
  - Crospovidonum; Crospopharm crosslinked povidone; E1202; Kollidon CL; Kollidon CL-M; Polyplasdone XL; PolyplasdoneXL-10; polyvinylpolypyrrolidone; PVPP; 1-vinyl-2-pyrrolidinone homopolymer.

- **Chemical name and CAS registry number**
  - 1-Ethenyl-2-pyrrolidinone homopolymer [9003-39-8]

- **Empirical formula and molecular weight**
  - \((\text{C}_6\text{H}_9\text{NO})_n \geq 1000000\)

- **Structural formula**

- **Functional category**
  - Tablet disintegrant

- **Description**
  - Crospovidone is a white to creamy-white, finely divided, free-flowing, practically tasteless, odorless or nearly odorless, hygroscopic powder.

- **Applications in pharmaceutical formulation or technology**
  - Crospovidone is a water insoluble tablet disintegrant and dissolution agent used at 25% concentration in tablets prepared by direct compression or wet and dry granulation methods. Larger particles provide a faster disintegration than smaller particles. Crospovidone can also be used as a solubility enhancer. With the technique of co-evaporation, crospovidone can be used to enhance the solubility of poorly soluble drugs. The drug is adsorbed on to crospovidone in the presence of a suitable solvent and the solvent is then evaporated. This technique results in faster dissolution rate (Rowe et al., 2009).