Liver is often abused by environmental toxins, poor eating habits, alcohol and over-the-counter drug use, that damage and weaken the liver leading to important public health problems like hepatitis, cirrhosis and alcoholic liver diseases (Treadway, 1998). These toxins generally lead to pathologically distinct liver diseases in clinical practice, any or all of the three conditions can occur together at the same time, in the same patient. These three conditions are fatty liver, hepatitis, and cirrhosis.

EXPERIMENTAL INDUCTION OF HEPATOTOXICITY

Ethanol

Acute or chronic administration of large amounts of ethanol causes morphological damage not only to the liver but also to many other organs, together with various metabolic changes, such as accumulation of triglycerides in the liver (Mezey, 1976). More than 60% of ethanol is rapidly oxidized in hepatocytes to acetate and then to carbon dioxide and water: The accompanying obligatory reduction of NAD to NADH alters the hepatic intracellular redox state, which can adversely affect many other metabolic reactions in the liver. These biochemical changes may contribute to the hepatotoxicity of ethanol (Utne and Winkler, 1980).
Galactosamine

Galactosamine-induced oxidative damage is generally attributed to the formation of the highly reactive hydroxyl radical (OH•), the stimulator of lipid peroxidation and the source of destruction and damage to the cell membrane (Barry and Gutteridge, 1989). This amino sugar is known to selectively block hepatic transcription and indirectly hepatic protein synthesis (Decker and Keppler, 1974).

Paracetamol

Acetaminophen and paracetamol, is usually well tolerated in prescribed dose but overdose is the most common cause of drug induced liver disease and acute liver failure worldwide (Keeffe et al., 2004). Damage to the liver is not due to the drug itself but to a toxic metabolite (N-acetyl-p-benzoquinone imine NAPQI, or NABQI) which is produced by cytochrome P450 enzymes in the liver (James et al., 2003). In normal circumstances this metabolite is detoxified by conjugating with glutathione in phase 2 reaction. In overdose large amount of NAPQI is generated which overwhelm the detoxification process and lead to damage to liver cells. Nitric oxide also plays a vital role in inducing toxicity (Wallace, 2004).

Many hepatotoxicants require metabolic activation, particularly by the liver cytochrome P450 enzymes, to form reactive, toxic metabolites, which in turn cause liver injury in experimental animals and humans (Gonzalez, 1988). Hepatitis induced by these chemicals shows many metabolic and morphological aberrations in the liver of experimental animals.
similar to those observed in human viral hepatitis (Gu et al., 1991). Paracetamol and CCl₄-induced hepatic injuries are commonly used models for hepatoprotective drug screening (Plaa and Hewitt, 1982).

**CARBON TETRA CHLORIDE INDUCED LIVER DAMAGE**

Carbon tetra chloride (CCl₄), a non-inflammable liquid with a characteristic odour is a potent and well-established hepatotoxin. Liver injuries induced by CCl₄ are the best-characterized system of the xenobiotic-induced hepatotoxicity and is a commonly used model for the screening the anti-hepatotoxic/hepatoprotective activity of drugs (Brautbar and Williams, 2002). By its administration chronic liver injury occurs along with fibrotic changes in the anatomy of the liver, degeneration and necrosis of liver cells, fibrous tissue proliferation and fatty liver production which constricts the blood flow in the liver sinusoids. There is also accumulation of interstitial fluids in the liver cells. CCl₄ is toxic to the liver and its toxicity depends on dose and time of exposure (Junnila et al., 2000). CCl₄, a well-known model compound for producing chemical hepatic injury, requires biotransformation by the hepatic microsomal cytochrome P-450 in the endoplasmic reticulum to produce hepatotoxic metabolites, namely trichloromethyl free radicals (•CCl₃ and/or *CCl₃OO) (Brattin et al., 1985; Williams and Burk, 1990; Brent and Rumack, 1993). The cytochrome P-450 system is encased in phospholipids membrane rich in polyenoic fatty acid. Hence these polyenoic fatty acids are the most likely immediate target for the initial lipid peroxidative attack to occur. The organic fatty acid radical rearranges, yielding organic peroxy and hydroxyl peroxy radicals. The radical destroy the cytochrome P-450
homoprotein, thus compromising the mixed – function oxygenase activity. The rapid decomposition of the endoplasmic reticulum and its function as a direct result of this lipid peroxidative process was observed in CCl₄ damage (Zangar et al., 2000).

Trichloromethyl free radicals can react with sulfhydryl groups, such as glutathione (GSH) and protein thiols, and the covalent binding of the trichloromethyl free radicals to the cell proteins is considered to be the initial step in a chain of events that eventually lead to membrane lipid peroxidation and finally to cell necrosis (Recknagel et al., 1991). Although several isoforms of P-450 can metabolize carbon tetrachloride, most attention has been focused on the P-450 2E1 isoform, which is ethanol inducible (Koop, 1992). Moreover, alterations in the activity of P-450 2E1 can affect the susceptibility to hepatic injury by CCl₄ (Jeong, 1999). P450 2E1 is active in the metabolism of small organic molecules including acetaminophen, aliphatic organic alcohols, nitrosamines, benzene, phenol, 4-nitrophenol, and pyrazole (Lee et al., 1996).

The reactive intermediates formed during the metabolism of therapeutic agents, toxins, and carcinogens by this enzyme are frequently capable of covalently binding to the tissue macromolecules, which result in tissue damage (Guengerich et al., 1991; Eaton et al., 1995). Hence, the suppression of P-450 can result in a reduction in the level of the reactive metabolites, and correspondingly, less tissue injury. Several studies have reported substances that influence the activity of P-450 2E1 and modulate the drug-induced hepatotoxicity. In particular, the compounds that induce
P-450 2E1 potentiate the hepatic toxicity of CCl₄ (Allis et al., 1996). On the other hand, compounds that inhibit P450 2E1 protect against CCl₄-induced toxicity (Kim et al., 1997). The induction or inhibition of CCl₄ biotransformation may subsequently influence the metabolic activation or detoxification of CCl₄.

In contrast to the toxic activation of CCl₄ via the P-450 2E1 pathway, the detoxification pathway involves GSH conjugation of the trichloromethyl radical, a P-450 2E1-mediated CCl₄ metabolite. Previous studies on the mechanism of CCl₄-induced hepatotoxicity have shown that GSH plays a key role in detoxifying the reactive toxic metabolites of CCl₄ and that liver necrosis begins when the GSH stores are markedly depleted. GSH is largely mediated through the activity of glutathione-S-transferase, and forms adducts with the toxic metabolites of CCl₄. Moreover, GSH contribute to the detoxification of CCl₄, and it has been suggested that one of the principal causes of CCl₄-induced liver injury is lipid peroxidation caused by its free radical derivatives (Recknagel et al., 1991).

Glutathione-S-transferase is a soluble protein located in the cytosol, and plays an important role in the detoxification and excretion of xenobiotics. The glutathione-S-transferase functionally binds GSH. Since it increases the solubility of hydrophobic substances, it also plays an important role in the storage and excretion of xenobiotics. Compounds that increase the glutathione-S-transferase activity, and metabolize toxic compounds to non-toxic compound, protect the liver (Boyer et al., 1984).
CCl₄ Toxicity and Lipid peroxidation

Several mechanisms have been proposed for CCl₄ induced fatty liver and necrosis. Important mechanisms include damage to endoplasmic reticulum, mitochondrial lysosomes, disturbances in hepatocellular calcium homeostasis, and lipid peroxidation. All are mediated by free radicals. Lipid peroxidation may be looked upon as occurring in two steps. Some toxic event initiates lipid peroxidation and organic free radical generated by the initiation process serve to propagate the reaction. CCl₄-induced liver dysfunction in rats simulates liver cirrhosis in man (Pérez-Tamayo, 1983; Wensing et al., 1990).

MECHANISM OF LIPID PEROXIDATION

- Fatty acid with three double bonds
- Hydrogen abstraction by Hydroxyl radical
- Unstable carbon radical
- Molecular Rearrangement
- Conjugated diene
- Oxygen uptake
- Peroxyl radical
- Hydrogen abstraction ↔ Chain reaction
- Lipid hydroperoxide
- malondialdehyde
- 4-hydroxynonenal
- ethane/pentane
- etc.
The steps involved in lipid peroxidation are described below and shown schematically (Anjali et al., 2001).

**Initiation:**

\[
\begin{align*}
H_2O & \rightarrow HO^\bullet, H^\bullet, e^-_{aq}, O_2^\bullet, H_2O_2 \\
LH^+ & \rightarrow L^\bullet + H_2O
\end{align*}
\]

**Propagation:**

\[
\begin{align*}
L^\bullet + O_2 & \rightarrow LOO^\bullet \\
LOO^\bullet + LH & \rightarrow LOOH + L^\bullet
\end{align*}
\]

**Termination:**

\[
\begin{align*}
L^\bullet + L^\bullet & \rightarrow L-L \\
LOO^\bullet + LOO & \rightarrow LOOH + O_2 \\
LOO^\bullet + L^\bullet & \rightarrow LOOL
\end{align*}
\]

Malondialdehyde is the major reactive aldehyde resulting from the peroxidation of biological membrane poly unsaturated fatty acid (PUFA). Thus MDA is used as an indicator of tissue damage and reacts with thiobarbituric acid and produce red colored products.

Hepatotoxicity following acute exposure to CCl₄ is manifested as necrosis and inflammation mainly in the centrilobular areas of the rodent liver (Germano et al., 2001). The covalent binding of the radical to cell components initiates the inhibition of lipoprotein secretion and thus steatosis, whereas reaction with oxygen, to form CCl₃-OO, initiates lipid peroxidation.
The latter process results in loss of calcium homeostasis and, ultimately, apoptosis and cell death (Boll et al., 2001). The massive production of reactive species may lead to depletion of protective physiological moieties (glutathione and α-tocopherol, etc.), ensuing widespread propagation of the alkylation as well as peroxidation, causing damage to the macromolecules in vital biomembranes (Aldridge, 1981). The reactive species mediated hepatotoxicity can be effectively managed upon administration of such agents possessing anti-oxidants, free radical scavengers and anti-lipid peroxidants (Sadanobu et al., 1999; Attri et al., 2000; Lim et al., 2000).

TRADITIONAL SYSTEM OF MEDICINES

Natural products, including plants, animals and minerals have been the basis of treatment of human diseases. History of medicine dates back practically to the existence of human civilization. The current accepted modern medicine or allopathy has gradually developed over the years by scientific and observational efforts of scientists. However, the basis of its development remains rooted in traditional medicine and therapies. Ayurveda remains one of the most ancient and yet living traditions practised widely in India, Sri Lanka and other countries and has a sound philosophical and experiential basis. Atharvaveda (around 1200 BC), Charak Samhita and Sushrut Samhita 26 (1000–500 BC) are the main classics that give detailed descriptions of over 700 herbs (Dahanukar and Thatte, 2000; Chopra and Doiphode, 2002).
Indian healthcare consists of medical pluralism and ayurveda still remains dominant compared to modern medicine, particularly for the treatment of a variety of chronic disease conditions. India has about 45,000 plant species, and medicinal properties have been assigned to several thousands. About 2000 are found in the literature; indigenous systems commonly employ about 500–700. The Government of India has formal structures to regulate quality, safety, efficacy and practice of herbal medicine. With unique holistic approach, ayurvedic medicines are usually customized to an individual constitution. The patient is not first treated for his symptoms, but the ingredients in the formulation are such that they go to the root of the problem bearing in mind, the patient’s basic constitutional requirements. Ayurveda is based on the balance and counter balance. No single principle has the activity of the whole. Some components potentiate a desired therapeutic action, while others reinforce the same and yet other interact to neutralize and counteract any possible side effect that might arise.

Exhaustive information is available in ayurvedic literature that can be converted into a large database giving information of various foods, herbs, medicines and other materials with their taste, actions and utility in different disorders. An innovative method to provide quantitative representations of various ayurvedic concepts, including, Prakruti, Rasa and Guna has been developed by the Indian Institute of Chemical Technology, Hyderabad. This patented technology has been registered as Herboprint and essentially gives a three dimensional HPLC fingerprint with Ayurvedhic property profile (National Policy on Indian Systems of Medicine and Homoeopathy, 2002).
The future of natural products drug discovery will be more holistic, personalized and involve wise use of ancient and modern therapeutic skills in a complementary manner so that maximum benefits can be accrued to the patients and the community (Patwardhan and Hooper, 1992; Steven King, 1992).

Combining the strengths of the knowledge base of traditional systems such as ayurveda with the dramatic power of combinatorial sciences will help in the generation of structure–activity libraries. Ayurvedic knowledge and experiential database can provide new functional leads to reduce time, money and toxicity – the three main hurdles in drug development. These records are particularly valuable, since effectively these medicines have been tested for thousands of years on people.

LIVER DISEASES AND MEDICINAL PLANTS

Polyherbal formulations reputed to have hepatoprotective activity that are available in the Indian market which comprise about one hundred Indian medicinal plants (Handa and Sharma, 1986).

*Andrographis paniculata*

For centuries Andrographis has been an important herb in the Asian healing systems of Ayurveda, Unani and Traditional Chinese Medicine. Traditionally the herb has been used to potentiate immune system response to inflammation and infections, and as an anti-inflammatory, antipyretic (lowers fevers) and a hepatoprotective. Andrographolide, the active constituent isolated from the plant *Andrographis paniculata* showed a significant dose
dependent protective activity against paracetamol-induced toxicity on \textit{ex vivo} preparation of isolated rat hepatocytes (Visen \textit{et al.}, 1993).

\textbf{Phyllanthus amaris}

\textit{Phyllanthus amaris} has been researched for its effects on hepatitis, and (Thyagaran \textit{et al.}, 1988) reported that 22 of 37 cases of Hepatitis B lost their "carrier" status after using the herb for a month.

\textbf{Boerhavia diffusa (Punarnava)}

An alcoholic extract of whole plant \textit{Boerhavia diffusa} given orally exhibited hepatoprotective activity against experimentally induced carbon tetrachloride hepatotoxicity in rats and mice (Chandan \textit{et al.}, 1991).

\textbf{Swertia chirata (Chirayata)}

Mukherjee \textit{et al.} (1997) reported that simultaneous treatments with \textit{S. chirata} (in different doses, viz., 20, 50, and 100 mg/kg body wt daily) and \textit{CCl}_4 caused improvement at both biochemical and histopathological parameters compared to that of \textit{CCl}_4 treatment alone but it was most effective when \textit{S. chirata} was administered in a moderate dose (50 mg/kg bodywt).

\textbf{Terminalia belerica (Baheda)}

Compound I isolated from fraction TB5 of \textit{Terminalia belerica} identified as 3,4,5-trihydroxy benzoic acid (gallic acid) and was evaluated for its hepatoprotective activity against carbon tetrachloride-induced physiological and biochemical alterations in the liver. Administration of
compound led to significant reversal of majority of the altered parameters confirming the presence of hepatoprotective activity (Anand et al., 1997).

**Cichorium intybus**

*Cichorium intybus* is a popular Ayurvedic medicine for the treatment of liver diseases. It is commonly known as kasni and is part of polyherbal formulations used in the treatment of liver diseases. In mice, liver protection was observed at various doses of *Cichorium intybus* but optimum protection was seen with a dose of 75 mg/kg given 30 minutes after CCl₄ intoxication. Kalantari and Rastmanesh (1985) in his preclinical studies showed that an alcoholic extract of the *Cichorium intybus* was found to be effective against chlorpromazine-induced hepatic damage in adult albino rats. A bitter glucoside, Cichorin (C₃₂H₄₄O₁₉) has been reported to be the active constituent of the herb.

**Glycyrrhiza glabra**

*Glycyrrhiza glabra*, commonly known as licorice contains triterpene saponin, known as glycyrrhizin, which has potential hepatotprotective activity. It belongs to a group of compounds known as sulfated polysaccharides. Experimental hepatitis and cirrhosis studies on rats found that it can promote the regeneration of liver cells and at the same time inhibit fibrosis. Glycyrrhizin can alleviate histological disorder due to inflammation and restore the liver structure and function from the damage due to carbon tetrachloride. The effects including: lowering the SGPT, reducing the degeneration and necrosis and recovering the glycogen and RNA of liver
cells. Effects of glycyrrhizin has been studied on free radical generation and lipid peroxidation in primary cultured rat hepatocytes. Favourable results have been reported in children suffering from cytomegalovirus after treating with glycyrrhizin (Numazaki et al., 1994).

**Curcuma longa**

*Curcuma longa* turmeric has been found to protect animal livers from a variety of hepatotoxic substances, including carbon tetrachloride, galactosamine, pentobarbitol, 1-chloro-2,4-dinitrobenzene, 7,4-hydroxy-nonenal, and paracetamol (Srinivas et al., 1991; Selvam et al., 1995). Diarylhepatonoids is the active constituent of the plant.

**Silimarín**

Silimarín is widely used as a standard hepatoprotective drug in many liver disorders. The main effects of silymarín are the membrane stabilising and antioxidant effects, help the liver cell regeneration, decrease the inflammatory reaction, inhibit the fibro genesis in the liver and the long administration of silymarín significantly increased the survival time of patients with alcohol-induced liver cirrhosis. Fehér et al. (2008) reported the antioxidant, antiperoxidative effects might be important factors in the mechanism of hepatoprotective action of silymarín. Silymarín prevents to a considerable degree the increase of the serum enzymes (GOT, GPT, MDH, SDH, ICDH, ACP. ACE activity caused by a D-galactosamine injury, enhances the metabolic conversion of the UDP-hexosamine into UDP-acetylhexosamine in the liver and hastens the normalizing of the UDP-
Silymarin corrected the altered immunoreactions and the decreased superoxid-dismutase (SOD) activity of erythrocytes and lymphocytes in patients with alcoholic liver cirrhoses. The scavenger effect silymarin was demonstrated in the sub cellular fractions of liver cells in animal experiments (Fehér et al., 1989).

*Melia azedarach*

Many studies have been performed with an antiviral compound isolated from the leaves of *Melia azedarach* L. named meliacine. Meliacine strongly inhibited the replication of HSV-1 and HSV-2 in Vero cells (Andrei et al., 1994) and exhibits a synergistic antiviral activity when combined with acyclovir (Barquero et al., 1997). Studies performed by Alché et al., 2002 suggested that MA exerts the antiviral action on both synthesis of viral DNA and maturation and progress of HSV-1 on Viral cells. Moreover, meliacine is a weak inducer of tumor necrosis factor alpha (TNF-α) in murine macrophage cultures and causes a synergistic effect on the production of TNF-α induced by LPS Ellerman-Eriksen (1994).

The isolated constituents and n- hexane extracts of piper longum were found to show varying degree of antibacterial activity against all the tested bacteria (Lokhande et al., 2007). Administration of alcoholic extract of Piper longum (10 mg/dose/animal) as well as piperine (1.14 mg/dose/animal) could inhibit the solid tumor development in mice induced with DLA cells and increase the life span of mice bearing Ehrlich ascites carcinoma tumor to
37.3 and 58.8%, respectively. Administration of *Piper longum* extract and piperine increased the total WBC count to 142.8 and 138.9%, respectively, in mice (Sunila and Kuttan, 2004). Ethanol extract of *Piper longum* fruits and five crude fractions, petroleum ether (40-60), solvent ether, ethyl acetate, butanol and butanone were subjected to preliminary qualitative chemical investigations. The ethanolic extract and all other fractions were screened orally for hepatoprotective activity in adult Wistar rats. The ethanolic extract and butanol fraction have shown significant activity, lowering the serum enzymes glutamic oxaloacetic transaminase and glutamic pyruvic transaminase in rats treated with carbon tetrachloride when compared to control and Liv-52-treated rats (Jalalpure *et al.*, 2003).