1.1. Introduction

Liver is the most important organ, which plays a pivotal role in regulating various physiological processes in the body. It is involved in several vital functions, such as metabolism, secretion and storage. It has great capacity to detoxicate toxic substances and synthesize useful principles. Therefore damage to the liver inflicted by hepatotoxic agents is of grave consequences. Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative stress in liver. Liver diseases are mainly caused by toxic chemicals, excessive consumption of alcohol, infections and autoimmune disorders (Rosa et al., 2009).

The liver is a large, complex organ that is well designed for its central role in carbohydrate, protein and fat metabolism. It is the site where waste products of metabolism are detoxified through processes such as amino acid deamination, which produces urea. In conjunction with the spleen it is involved in the destruction of spent red blood cells and the reclamation of their constituents. It is responsible for synthesizing and secreting bile and synthesizing lipoproteins and plasma proteins, including clotting factors. It maintains a stable blood glucose level by taking up and storing glucose as glycogen (glycogenesis), breaking this down to glucose when needed (glycogenolysis) and forming glucose from noncarbohydrate sources such as amino acids (gluconeogenesis) (Burkitt et al., 1993).

Many of these biosynthetic functions use the products of digestion. With the exception of most lipids, absorbed food products pass directly from the gut to the liver through the hepatic portal vein. At the microscopic level, the primary functional unit of the liver is the liver acinus, which is defined by the territory supplied by each terminal branch of the hepatic artery and hepatic portal vein. The portal tract forms the central axis of the acinus; hepatocytes are arranged in plates that radiate out from the portal triad. The acinus is divided into 3 zones on the basis of the distance from the supplying vessels: zone 3, for example, experiences the least oxygen perfusion and houses the most mitochondria. Bile is secreted into a network of minute bile caniculi situated between adjacent hepatocytes (Worobetz et al., 1994). Thus to maintain a healthy liver is a crucial factor for overall health and well being.
Excessive production of reactive oxygen species (ROS) plays an important role in the pathogenesis and progression of various diseases involving different organs (Visioli et al., 2000). Lipid peroxides produced from unsaturated fatty acids via free radicals cause toxic effects and promote the formation of additional free radicals in a chain reaction. If the in vivo activity of enzymes or scavengers is not adequate to neutralize these radicals, oxidative stress develops and leads to various diseases such as cancer, cardiovascular diseases, diabetes mellitus, liver diseases, brain dysfunction, or accelerated aging may result (Karunakar et al., 2009). The rationale for the use of antioxidants is well established in prevention and treatment of diseases where oxidative stress plays a major etiopathological role. Antioxidants may protect the body against ROS toxicity either by preventing the formation of ROS, by the interruption of ROS attack, by scavenging the reactive metabolites or by converting them to less reactive molecules (Sen, 1995).

Medicinal plants form the backbone of traditional system of medicine in India. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds (Prusti et al., 2008). Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design. Medicinal plants are rich source of novel drugs that forms the ingredients in traditional system of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates, bioactive principles and lead compounds in synthetic drugs (Ncube, 2008). Liver diseases remain one of the serious health problems and the Indian traditional system of medicine, especially Ayurveda have put forward a number of medicinal plants and their formulations for liver disorder. In this modern age it is very important to provide scientific proof to justify the various medicinal uses of herbs. Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness and relatively low cost (Valiathan, 1998).

1.2. Alcoholic liver disease (ALD)

Alcoholic liver disease encompasses a spectrum of injury, ranging from simple steatosis to frank cirrhosis. It may well represent the oldest form of liver injury known to humankind. Alcohol remains a major cause of liver disease worldwide. Often, as people continue to drink heavily, they progress from fatty liver to hepatitis to cirrhosis. The disorders can also occur together and liver biopsies can show signs of all three in some people (Robert et al., 2003)
1.2.1. Alcoholic fatty liver

About 20 percent of alcoholics and heavy drinkers develop fatty liver, or steatosis. In many cases there are no clinical symptoms except for an enlarged liver (hepatomegaly). Fatty liver can be reversed if alcohol consumption is stopped or significantly reduced, but the condition can lead to death if alcohol consumption is not reduced or stopped. Some biopsies from people with fatty liver show inflammatory changes, an early sign of more serious liver disease (Hall, 1995).

1.2.2. Alcoholic Hepatitis

Alcoholic hepatitis usually is diagnosed when a liver biopsy indicates inflammatory changes, liver degeneration, fibrosis and other changes to liver cells. Common clinical signs of alcoholic hepatitis include swollen liver, nausea, vomiting and abdominal pain. Patients also may experience fever, jaundice, liver failure and bleeding. The rate of mortality in severe cases is about 50 percent. If heavy drinking continues about 40 percent of cases of alcoholic hepatitis will develop into cirrhosis (Corrao et al., 1998).

Fig.1 Different forms of Alcoholic Liver Disease

1.2.3. Alcoholic Cirrhosis

Cirrhosis of the liver is the most serious form of ALD and a cause of many deaths and serious illness. In cirrhosis, scar tissue replaces normal liver tissue, disrupting blood flow through the liver and preventing it from working properly.
Clinical signs of cirrhosis include redness of palms caused by capillary dilation (palmar erythema); shortening of muscles in the fingers (contractures) caused by toxic effects or fibrous changes; white nails; thickening and widening of the fingers and nails (clubbing); liver enlargement or inflammation; and abnormal accumulation of fat in normal liver cells (fatty infiltration). About 10 percent to 15 percent of people with alcoholism develop cirrhosis. Many are unaware that they have it, and about 30 percent to 40 percent of cirrhosis cases are discovered at autopsy (Anand 1999).

1.3. Pathways of alcohol metabolism

Alcohol is broken down in the liver primarily through two pathways: the alcohol dehydrogenase (ADH) pathway and the microsomal ethanol-oxidizing system (MEOS). In people who consume alcohol at moderate levels and/or only occasionally, most of the alcohol is broken down by ADH, an enzyme found in the cytosol. ADH converts alcohol to acetaldehyde, a toxic and highly reactive molecule. During this reaction, hydrogen is removed from the alcohol and transferred to a molecule called nicotinamide adenine dinucleotide (NAD), converting it to reduced NAD (NADH). NADH participates in numerous other metabolic reactions, passing on hydrogen to other compounds, and excess cellular NADH levels have harmful effects on those cells. Subsequently, the acetaldehyde is converted to acetate by a second enzyme, aldehyde dehydrogenase (Wu et al., 2006). The MEOS plays a role in alcohol metabolism, particularly after higher alcohol consumption. The MEOS occurs in microsomes, and the main component of the MEOS is the enzyme cytochrome P450, which like ADH, converts alcohol to acetaldehyde. This reaction also relies on oxygen and a molecule called nicotinamide adenine dinucleotide phosphate (NADPH) and results in the formation of NADP and water. Also, the combination of hydrogen peroxide generation from NADPH oxidase and of catalase was revealed to account for microsomal ethanol oxidation. In summary, the two ethanol oxidation pathways described above mainly generate acetaldehyde that causes further liver injury and toxicity (Wu et al., 2006).

Although the rate at which ADH breaks down alcohol generally stays the same, the activity of the MEOS can be increased by alcohol consumption. Because the MEOS metabolizes not only alcohol but also other compounds, (certain medications) enhanced MEOS activity resulting from high alcohol consumption also can alter the metabolism of those medications. This may contribute to harmful interactions between alcohol and those medications or otherwise influence the activity of those medications (Salmela et al., 1998).
Of the several variants of cytochrome P450, a form called CYP2E1 is most prominent in alcohol metabolism. The activity of this molecule can increase up to four-fold following alcohol consumption (Tsutsumi et al., 1989). Other types of cytochrome P450, such as CYP1A2 and CYP3A4, also are involved in the breakdown of alcohol (Salmela et al., 1998).

**Fig. 2 Pathways of Alcohol Metabolism**

\[
\begin{align*}
\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ & \xrightarrow{\text{ADH}} \text{CH}_3\text{CHO} + \text{NADH} + \text{H}^+ \\
\text{CH}_3\text{CH}_2\text{OH} + \text{NADPH} + \text{H}^+ + \text{O}_2 & \xrightarrow{\text{MEOS}} \text{CH}_3\text{CHO} + \text{NADP}^+ + 2\text{H}_2\text{O} \\
\text{NADPH} + \text{H}^+ + \text{O}_2 & \xrightarrow{\text{NADPH Oxidase}} \text{NADP}^+ + \text{H}_2\text{O}_2 \\
\text{H}_2\text{O}_2 + \text{CH}_3\text{CH}_2\text{OH} & \xrightarrow{\text{Catalase}} \text{CH}_3\text{CHO} + 2\text{H}_2\text{O} \\
\text{CH}_3\text{CHO} + \text{NAD}^+ & \xrightarrow{\text{ALDH}} \text{CH}_3\text{CHO} + \text{NAD} + \text{H}^+ \\
\end{align*}
\]

In addition, increased NADH promotes the generation of the building blocks of fat molecules (fatty acids) and reduces the breakdown of fats in the liver, thereby contributing to fat accumulation in that organ (Lieber and Schmid, 1961). Other alcohol-related mechanisms also contribute to fat accumulation in the liver, including: (i) Decreased excretion of fat-containing proteins from the liver, (ii) Release of fats from other tissues, which then are transported to the liver, (iii) Enhancement of the liver’s uptake of fats circulating in the blood. The resulting fatty liver is the earliest stage and the most common form of alcohol-induced liver disease.

In addition to contributing to the development of fatty liver, the increase in NADH levels resulting from the ADH-mediated breakdown of alcohol may play a role in the formation of scar tissue that characterizes fibrosis, a more severe stage of liver disease (Casini et al., 1991).
1.4.1. Pathophysiology of ALD

Recently, various proinflammatory cytokines have been proposed to play a role in this disease (Tilg et al., 2000). Among these cytokines, the proinflammatory cytokine TNFα has emerged as a key cytokine in the inflammatory process. The involvement of TNFα has been especially demonstrated in acute alcoholic hepatitis. Indeed, alcoholic hepatitis was one of the first diseases shown to exhibit increased circulating TNFα levels (Khoruts et al., 1991). In addition, serum concentrations of various TNF-inducible cytokines such as interleukin-1L-1 and 1L-8 are also increased in patients with acute alcoholic hepatitis (Hill et al., 1993). Furthermore, plasma levels of both TNF and soluble TNF receptors are correlated with endotoxemia and stage of liver disease (Hanck et al., 1998). This finding, coupled with evidence that long term ingestion of alcohol increases intestinal permeability and those patients with the highest serum concentrations have the highest in-hospital mortality (Felver et al., 1990), indicates that intestinally derived endotoxin and endotoxin-regulated cytokines such as TNFα, have a role in the pathogenesis of alcoholic steatohepatitis (Adachi et al., 1995).

Human data supporting a key role for TNFα in alcohol-related liver diseases are further substantiated by data from animal experiments (Yin et al., 1999). Several studies in rats, mice and tissue culture focused on the role of cytokines especially TNFα, in experimental models of alcoholic liver disease (Tilg et al., 2000). Perhaps the most compelling evidence supporting a key role for this cytokine comes from studies using mice in which the type-1 TNF receptor gene has been disrupted as these mice are resistant to alcohol-induced liver disease (Yin et al., 1999). Anti- TNF antibody treatment has also been successfully used to prevent liver in alcohol-fed rats (Iimuro et al., 1997). Furthermore, alcohol-associated liver injury is inhibited when the animals are treated with poorly absorbed oral antibiotics or lactobacillus to decrease endotoxemia, supporting the hypothesis that gut-derived bacterial products such as endotoxin might be important in activation of Kupffer cells and/or other cell types in the liver. This is in accordance with the recent observations that chronic ethanol feeding causes more severe liver injury in wild-type mice than in CD 14 knockouts (Yin et al., 2001). These results further support the notion that gut-derived endotoxin acting via its cellular receptor CD14 plays a major role in the development of early alcohol-induced liver injury.
1.4.2. Apoptosis and ALD

Apoptosis, a major form of cell death, has been noted in a diverse spectrum of liver diseases. In experimental models and isolated hepatocytes, alcohol has been shown to induce liver cell apoptosis. Recently, two reports demonstrated that enhanced apoptosis is a prominent feature of alcoholic steatohepatitis (Natori et al., 2001).

1.4.3. Epidemiology

One of the most enduring insights into the effects of alcohol has been the assertion that heavy alcohol consumption increases mortality rates, especially those from cirrhosis of the liver and other forms of liver disease. The scientific study of alcohol-related mortality began in the 1920s with Pearl’s studies (1926) of death rates among various types of drinkers.

He and others found that heavy drinkers had higher rates of overall mortality and of mortality from cirrhosis than the lighter drinkers or abstainers. Since then, mortality studies have continued to demonstrate that heavy drinkers and alcoholics die from cirrhosis at a much higher rate than the general population (Thun et al., 1997). In addition, laboratory studies conducted in the 1930s established that feeding large amounts of alcohol to rats and other animals caused liver disease (Lelbach, 1974).

Alcohol consumption increased substantially in many countries after World War II, which spurred greater interest in the effects of alcohol consumption on cirrhosis and other forms of ALD. One of the most influential efforts to summarize research in this area was undertaken in 1975 by an international group of scientists sponsored by WHO. The resulting book, Alcohol Control Policies in Public Health Perspectives (Bruun et al, 1975), reviewed studies of clinical and nonclinical populations of heavy drinkers. All studies found that a greater proportion of heavy drinkers died of cirrhosis than would be expected based on rates of cirrhosis deaths in the general population.

This research established a firm connection between heavy alcohol consumption and liver disease. Investigators also have observed that the price of alcohol is a significant determinant of alcohol consumption and thus of cirrhosis mortality rates (Bruun et al., 1975; Edwards et al., 1994; Selley, 1960). These findings have laid the foundation for an influential public health approach to controlling liver disease and other alcohol problems that emphasizes the control of alcohol’s availability and
includes recommendations to control cirrhosis and other alcohol-related problems through taxation (Chaloupka et al., 2002; Cook and Tauchen 1982). The validity of this availability-control approach has been widely supported (Edwards et al., 1994), and investigations of the epidemiology of ALD have continued to be central to it (Ramstedt, 2001).

1.4.4. Etiology of ALD

A complex interplay exists between a person’s alcohol consumption and nutritional status. Many people, including light-to-moderate drinkers who consume one to two glasses or less of an alcoholic beverage per day, consider those beverages a part of their normal diet and acquire a certain number of calories from them. When consumed in excess, however, alcohol can cause disease by interfering with the nutritional status of the drinkers. For example, alcohol can alter the intake, absorption into the body and utilization of various nutrients. In addition, alcohol exerts some harmful effects through its breakdown and the resulting toxic compounds particularly in the liver, where most of the alcohol metabolism occurs (Lieber, 1992, 2000).

Alcoholic beverages primarily consist of water, pure alcohol (ethanol) and variable amounts of sugars (carbohydrates); their content of other nutrients (proteins, vitamins or minerals) is usually negligible. Therefore, any calories provided by alcoholic beverages are derived from the carbohydrates and alcohol they contain. The carbohydrate and alcohol content vary greatly among the types of beverages. However, alcohol-derived calories when consumed in substantial amounts can have less biologic value than carbohydrate-derived calories (Pirola and Lieber., 1972). Some of the energy contained in alcohol is ‘lost’ or ‘wasted’ and it is not available to the body for producing or maintaining body mass. Several mechanisms have been implicated in the apparent loss of alcohol-derived energy. For example, some of the energy may be used up (wasted) during the breakdown of alcohol by a pathway known as the MEOS (Feinman and Lieber, 1998). As by products of these reactions, highly reactive oxygen-containing molecules called oxygen radicals or ROS are generated. These ROS can contribute to liver damage through a variety of mechanisms (Leo et al., 1993).

Alcohol can also interfere with the uptake of the essential amino acids indeed, studies using experimental animals have found that the animals absorb less amino acid from the intestine after they received an alcohol dose (Adibi et al., 1992).
1.4.5. Risk factors

A number of risk factors have been identified that influence the risk of development and progression of liver disease. Possible factors that affect the development of liver injury include the dose, duration, type of alcohol consumption, drinking patterns, sex, ethnicity and associated risk factors including obesity, iron overload, concomitant infection with viral hepatitis and genetic factors.

The amount of alcohol ingested (independent of the form in which it is ingested) is the most important risk factor for the development of ALD (Savolainen et al., 1993). A significant correlation exists between per capita consumption and the prevalence of cirrhosis (Ramstedt, 2001). The risk of developing cirrhosis increases with the ingestion of >60-80g/day of alcohol for 10 years or longer in men, and >20g/day in women (Bellentani et al., 1997).

The type of alcohol consumed may influence the risk of developing liver disease. In a survey of more than 30,000 persons in Denmark, drinking beer or spirits was more likely to be associated with liver disease than drinking wine (Becker et al., 2002).

Another factor that has been identified is the pattern of drinking. Drinking outside of meal times has been reported to increase the risk of ALD by 2.7 fold compared to those who consumed alcohol only at meal times (Lu et al., 2004). Binge drinking, defined by some researchers as five drinks for men and four drinks for women in one sitting, has also been shown to increase the risk of ALD and all cause of mortality (Barrio et al., 2004).

Women have been found to be twice as sensitive to alcohol-mediated hepatotoxicity and may develop more severe ALD at lower doses and with shorter duration of alcohol consumption than men (Sato et al., 2001). Several studies have shown differing blood alcohol levels in women versus men after consumption of equal amounts of alcohol (Baraona et al., 2001). This might be explained by differences in the relative amount of gastric alcoholdehydrogenase, a higher proportion of body fat in women, or changes in alcohol absorption with the menstrual cycle (Frezza et al., 1990).

A higher risk of liver injury may be associated with an individual’s racial and ethnic heritage (Stewatt, 2002). The rates of alcoholic cirrhosis are higher in African-
American and Hispanic males compared to Caucasian males and the mortality rates are highest in Hispanic males (Stinson et al., 2001). These differences do not appear to be related to differences in amounts of alcohol consumed (Wickramasinghe et al., 1995).

The presence and extent of protein calorie malnutrition play an important role in determining the outcome of patients with ALD. Mortality increases in direct proportion to the extent of malnutrition, approaching 80% in patients with severe malnutrition (i.e., less than 50% of normal) (Mendenhall et al., 1995). Micronutrient abnormalities, such as hepatic vitamin A depletion or depressed vitamin E levels, may also potentially aggravate liver disease (Leevy et al., 2005). Obesity and excess body weight have been associated with an increased risk of ALD (Naveau et al., 1997).

There is a clear synergistic relationship between chronic viral hepatitis and alcoholism resulting in more advanced liver disease jointly than separately. The combination of hepatitis C virus and alcohol predisposes to more advanced liver injury than alcohol alone (Monto et al., 2004).

In addition to environmental factors, genetic factors predispose to both alcoholism and ALD (Uhl et al., 2001). Children of alcoholism raised in adopted families had a significantly higher rate of alcohol dependence than did adopted children of nonalcoholics, who served as controls (18% versus 5%) (Goodwin et al., 1973). In population based studies, monozygotic twins were approximately twice as likely to drink as dizygotic twins; among those who drank, monozygotic twins were more likely to have a similar frequency and quantity of alcohol consumption (Kaprio et al., 1987). Moreover, monozygotic twins have a significantly higher prevalence of alcoholic cirrhosis than do dizygotic twins (Reed et al., 1996). Finally, polymorphism of genes involved in the metabolism of alcohol (including alcohol dehydrogenase, acetaldehyde dehydrogenase and the cytochrome P450 system), and in those which regulate endotoxin-mediated release of cytokines have been associated with ALD (McClain et al., 2004).

1.4.6. Prognosis in ALD

Decision regarding treatment is critically dependent on the ability to estimate a given patient’s prognosis. Many individual clinical and laboratory features, along with specific histologic features have also been tested as measures of disease prognosis. In
AH, the Maddrey discriminant function (MDF), a disease-specific prognostic score, has been used to stratify a patient’s severity for illness (Maddrey et al., 1978). The initial formula was derived in the context of clinical trials of alcoholic hepatitis, and later modified to: MDF=4.6 [(Patient’s prothrombin time-control prothrombin time) + total bilirubin (mg/dL)] (Carithers et al., 1989). Patients with a score of greater than or equal to 32 were at the highest risk of dying, within month mortality as high as 30%-50% (Mathurin et al., 1996). In particular those with evidence of both hepatic encephalopathy and an elevated MDF were at highest risk. Although relatively easy to use, and based on standard laboratory test, several drawbacks to the use of the MDF have been noted. Although it is a continuous measure, its interpretation (using a threshold of 32) has converted it into an essentially categorical method of classification. Once patients have exceeded that threshold their risk for dying is higher, but not specified. Dynamic models, which incorporate the changes in the laboratory studies over time have also been used to estimate the outcome in patients, including the change in bilirubin in the first week of hospitalization, which is significantly associated with outcome of patients with alcoholic hepatitis treated with prednisolone (Mathurin et al., 2003).

Other scoring systems also been proposed to stratify patients, including the combined clinical and laborotary index of the University of Toronto (Orrego et al., 1983) the Beclere model (Mathurin et al., 1996), the MELD (Model for End-Stage Liver Disease) score (Kamath et al., 2001) and the Glasgow Alcoholic Hepatitis Score (GAHS) (Forrest et al., 2005). The diagnostics abilities if the latter two models have been tested against the MDF and other scoring systems for cirrhosis (such as the Chid-TurcottePugh score, or CTP) in terms of specific test characteristics, including sensitivity and specificity, atleast in some populations (Sheth et al., 2002). Because of the inherent trade-off involved in setting test thresholds, optimal cut points are not clearly established for each of these indices. Some investigators have suggested specific cut offs for these indices, including an MDF ≥32 or a MELD score >11, that appear to be roughly equivalent in ability to detect patients with a poor prognosis, with similar sensitivity and specificity (Sheth et al., 2002).

Several studies have also demonstrated the utility of repeat testing and calculation of these indices during the course of hospitalisation including MELD or MDF score at one week, and degree of change. A change of ≥2 points in the MELD
score in the first week as been shown to independently predict in-hospital mortality (Srikureja et al., 2005). The GAHS was recently derived and its test characteristics compared to the MDF and MELD scores. Although it had an overall higher accuracy, it was substantially less sensitive for predicting one month and three months mortality compared to either the MDF or the MELD (Forrest et al., 2005). The degree of portal hypertension may be a sensitive marker for the severity of liver injury (Rincon et al., 2007). A recently proposed scoring system combines measurements of a marker of portal hypertension, asymmetric dimethylarginine and its stereoisomer, to predict outcomes (Mookerjee et al., 2007). This combined score has been compared to the CTP score, MELD and MDF, and shown to have an overall sensitivity of 73% and specificity of 83% which was at least as good as other scoring systems.

1.4.7. Treatment for ALD

Alcohol consumption and alcohol metabolism can lead to harmful effects on the liver through numerous pathways related to the drinker’s nutrition and metabolism. ALD develops in several sequential and partially overlapping stages. The first stage, fatty liver is characterized by fat accumulation in the liver; it is sometimes associated with inflammation, and is called steatohepatitis or alcoholic hepatitis, when severe. At this stage, liver cells may begin to die and scar tissue may form, leading to the next stage of liver disease, fibrosis. Excessive scar tissue formation, in turn, eventually destroys the normal liver structure, resulting in cirrhosis, the most severe type of liver disease.

Treatment of ALD must be started as early as possible in the disease process because patients are more likely to die as the disorder advances. For example a study of patients with ALD found that 70% of the patients with fatty liver still were alive after 4 years, whereas less than 50% of the patients with cirrhosis still were alive after the same amount of time. If the cirrhosis was associated with inflammation (alcoholic hepatitis), the outlook was even worse, with only about 33% of patients still alive after four years (Chedid et al., 1991). Unfortunately, these high mortality rates, higher than those for many cancers, attract relatively little attention from the public because many people believe that no effective treatment of ALD is available. However, new insights into the mechanisms contributing to the disorder have resulted in prospects for improved treatments, including nutritional management approaches that can lead to better outcomes.
(i) **Nutritional Therapy (Diet with MCT)**

Consumption of fat molecules known as long-chain triglycerides promotes fatty liver, whereas medium-chain triglycerides (MCTs) significantly reduce alcoholic fatty liver. This difference probably results from the fact that MCTs are more likely to be broken down in the body than long-chain triglycerides and therefore are less likely to be deposited in the liver (Lieber et al., 1967). Animal studies have confirmed that MCTs can protect against fat deposition in the liver (Nanji et al., 1996).

(ii). **Antioxidant therapy**

GSH is a small molecule consisting of three amino acids, including cysteine. Acetaldehyde, the first product of alcohol breakdown, can bind to GSH and specifically to cysteine, thereby removing active GSH from the liver cells (Shaw et al., 1983).
Because GSH depletion plays a key role in alcoholic liver injury, it is therapeutically important to increase GSH levels in the liver. GSH cannot be administered directly, however, because the molecule cannot penetrate directly into the liver cells. Similarly, the amino acid cysteine, which is most important for ensuring adequate GSH levels, cannot be used as a supplement because it cannot enter the liver cells. Therefore, precursors of cysteine, such as the compound acetylcysteine or S-adenosylmethionine can be administered, which can reach the cells and can be converted to cysteine (Vendemiale et al., 1989).

(iii) Therapy with PPC

One of the harmful consequences of alcohol breakdown by the MEOS is the formation of ROS, which among other effects can cause lipid peroxidation (LPO). Polyenylphosphatidylcholine (PPC), which is the mixture of molecules known as phosphatidylycerolines, prevented LPO (Aleynik et al., 1997) and attenuated the associated liver injury in rats that had been treated with hepatic toxins (Ma et al., 1996).

Therapy with Silymarin

Another antioxidant that has shown positive results in experimental animals (Lieber et al., 2003) is a molecule called silymarin, the active constituent of milk thistle. Some clinical trials have shown that this compound has beneficial effects such as improved survival in patients with ALD (Ferenci et al., 1989).

Anti-inflammatory therapy

Because of the potential role of inflammatory factors in the pathogenesis of fibrosis and cirrhosis, anti-inflammatory therapy with corticosteroids has been used. It improved survival rates in encephalopathic patients but not in those with milder illness (Carithers, 1989). Colchicine has also been evaluated as a treatment for alcoholic cirrhosis because of its anti-inflammatory and antifibrotic effect (Kershenobich et al., 1988).

1.5.1. Hepatic marker enzyme

The diagnosis of ALD is made by documentation of alcohol excess and evidence of liver disease (Menon et al., 2001). No single laboratory marker definitively establishes alcohol to be the etiology of liver disease. A wide variety of biochemical parameters are affected by regular excessive alcohol consumption. Abnormal liver enzyme levels may signal liver damage. The blood tests traditionally used most
commonly as markers of recent drinking of alcohol are the liver enzymes, gamma glutamyltransferase (GGT), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP). GGT is a glycoprotein enzyme situated on the cell membrane in several tissues. It is possibly involved in reabsorption of glutathione from the glomerular filtrate and in protection against oxidative stress via maintenance of intracellular glutathione levels (Whitfield, 2001). Clinically, it has been used as a measure of liver function or damage, but it is also found in the kidney, brain, spleen, pancreas and heart (Flanigan et al., 1996). Hepatic GGT levels increase in response to exposure to a variety of drugs and to alcohol. This may be mediated via oxidative stress, with resultant reductions in glutathione levels. Normally, small amounts of GGT are released from the cell membrane into the circulation. In people with repeated excessive alcohol consumption, there may be increased release of GGT from the cell membrane. In cases with inflammation and liver cell damage, there may also be cell necrosis with release of the enzyme (Katherine et al., 2003).

1.5.2. Amino transferases

A number of laboratory abnormalities, including elevated serum aminotransferases, have been reported in patients with alcoholic liver injury, and used to diagnose ALD (Nalpas et al., 1986). AST (SGOT) and ALT (SGPT) are sensitive indicators of liver cell injury. They are hepatocellular enzymes involved in amino acid metabolism. ALT is found predominantly in the cytosol, whereas AST activity is highest in the mitochondria. The enzymes reach the circulation via cell membrane. As with GGT, aminotransferases are often ordered as part of a battery of routine biochemical tests (Pratt et al., 2000). Alcohol is the most common cause of ALT elevation in otherwise healthy people. In a group of 100 blood donors with elevated ALT, alcohol was the most likely cause of elevation in half the cases, followed by obesity (22%), while 17-20% of donors were hepatitis C positive (Katkov et al., 1991). Like GGT, the aminotransferases act not only as markers of alcohol consumption but also as indicators of hepatic damage from alcohol. In one study 56% of subjects with ALT elevation had fatty liver on biopsy (Hulterantz et al., 1986).

AST is found in many body tissues including the heart, muscle, kidney, brain and lung. It is also present in the liver. When body tissue or an organ such as the heart or liver is damaged, additional AST is released into the bloodstream. The amount of
AST in the blood is directly related to the extent of tissue damage (Dufour et al., 2000). Mitochondrial AST is a specific isoform of the AST enzyme that is released from hepatocytes at particularly high levels in association with alcohol abuse (Lumeng, 1986). Although serum levels of both ALT and AST become elevated whenever disease processes affect liver cells, ALT is the most liver specific enzyme. Elevations of ALT activity persist longer than do those of AST activity (Johnson, 1999).

ALP is an enzyme that transports metabolites across cell membranes. Liver and bone diseases are the most common causes of pathological elevation of ALP levels, although ALP may originate from other tissue, such as the placenta, kidneys or intestines, or from leukocytes (Fishman, 1990). Hepatic ALP is present on the surface of bile duct epithelia. Cholestasis enhances the synthesis and release of ALP, and accumulating bile salts increase its release from the cell surface. (Moss, 1997; Schlaeger et al., 1982). Drug-induced liver injury may present with a cholestatic pattern the degree of ALP alteration is variable and may be accompanied by hyperbilirubinemia (Velayudham et al., 2003).

1.6.1. Free Radicals and ROS

A free radical is an atom, molecule, or compound that is highly unstable because of its atomic or molecular structure (i.e., the distribution of electrons within the molecule). As a result, free radicals are very reactive as they attempt to pair up with other molecules, atoms, or even individual electrons to create a stable compound. To achieve a more stable state, free radicals can steal a hydrogen atom from another molecule, bind to another molecule, or interact in various ways with other free radicals.

(i). Hydrogen abstraction, in which a radical interacts with another molecule that has a free hydrogen atom (a hydrogen donor). As a result, the radical binds to the hydrogen atom and becomes stable, whereas the hydrogen donor is converted to a free radical.

(ii). Addition, in which the radical binds to another, originally stable molecules, converting the combined molecule into a radical.

(iii). Termination, in which two radicals react with each other to form a stable compound.

(iv). Disproportionation, in which two identical radicals react with each other, with one of the radicals donating an electron to the other so that two different molecules are formed, each of which is stable.
One chemical element frequently involved in free radical formation is oxygen. Molecular oxygen \( (O_2) \) is essential for cell function because it plays a pivotal role in a series of biochemical reactions occurring in the respiratory chain, which is responsible for the most of the production of ATP, which provides the energy required for a multitude of cellular reactions and functions.

In the respiratory chain, which takes place in membrane-enclosed cell structures called mitochondria, an electron and a proton are removed from a cofactor called NADH (Adachi et al., 2002). The electron is transferred to the first component of respiratory chain, and the proton is released into the surrounding fluid. Chemically speaking, NADH is oxidised to \( \text{NAD}^+ \) in this reaction, whereas the respiratory chain component that accepts the electron is reduced (Bailey et al., 2002). The \( \text{NAD}^+ \) subsequently can be used again to accept new hydrogen atoms that are generated during the metabolism of sugars and other nutrients. The reduced respiratory chain component, in turn, passes the electron on to other molecules in the respiratory chain until it is finally transferred to \( O_2 \), which then interacts with protons in cells to generate water. This series of electron transfer reactions generates sufficient energy to produce several molecules of ATP for each electron that passes through the respiratory chain.

Molecular oxygen can accept a total of four electrons, one at a time, and the corresponding number of protons to generate two molecules of water. During this process, different oxygen radicals are successively formed as intermediate products, including superoxide \( (O_2^-) \); peroxide \( (O_2^+) \), which normally exists in cells as hydrogen peroxide \( (H_2O_2) \); and the hydroxyl radical \( (\cdot OH) \). Super oxide, peroxide, and the hydroxyl radicals are considered as the primary ROS and have sparked major research on the role of free radicals in biology and medicine (Bondy, 1992). These ROS formed as a natural by product of the normal metabolism of oxygen have important roles in cell signalling and homeostasis (Devasagayam et al., 2004).

However, because they are unstable and rapidly react with additional electrons and protons, most of these ROS are converted to water before they can damage cells. It has been estimated that only about 2 to 3 percent of the \( O_2 \) consumed by the respiratory chain is converted to ROS (Chance et al., 1979). Nevertheless, the toxic effects of oxygen in biological systems - such as the breakdown of lipids, inactivation of enzymes, DNA damage, and the destruction of cell membranes and, ultimately, cells are attributable to the reduction of \( O_2 \) to ROS (Toykuni 1999; Nakazawa et al., 1996).
While ROS are produced as a product of normal cellular functioning, excessive amounts can cause deleterious effects (Patel et al., 1999). Under certain conditions, such as acute or chronic alcohol exposure, ROS production is enhanced and/or the level or activity of antioxidants is reduced. The resulting state, which is characterized by a disturbance in the balance between ROS production on one hand and ROS removal and repair of damaged complex molecules (such as proteins or DNA) on the other is called oxidative stress (Halliwell, 1999).

**Fig. 4. Effects of ROS and Antioxidant Defense**

In general, harmful effects of reactive oxygen species on the cell are most often:

1. oxidations of polyunsaturated fatty acids in lipids (lipid peroxidation)
2. damage of DNA

**1.6.2. Lipid peroxidation**

Lipids that contain phosphate groups (i.e., phospholipids) are essential components of the membranes that surround the cells as well as other cellular structures, such as the nucleus and mitochondria. Consequently, damage to the phospholipids will compromise the viability of the cells. The complete degradation (i.e., peroxidation) of lipids is a hallmark of oxidative damage. The polyunsaturated fatty acids present in the membranes phospholipids are particularly sensitive to attack by hydroxyl radicals and other oxidants. Unsaturated fatty acids are those that contain a double bond between two of the carbon atoms making up the backbone of the fatty acid molecule. These double bonds can easily be opened in chemical reactions and interact with other substances. Fatty acids containing only one such double bond are called
monounsaturated; fatty acids with two or more double bonds are called polyunsaturated.) A single hydroxyl radical can result in the peroxidation of many polyunsaturated fatty acid molecules because the reactions involved in this process are part of a cyclic chain reaction. In addition to damaging cells by destroying membranes, lipid peroxidation can result in the formation of reactive products that themselves can react with and damage proteins and DNA. Oxidation of lipids also impairs cell-to-cell communication and nerve transmission, and oxidized lipids are the main culprits in atherosclerosis. Vitamin E is the major lipid-soluble antioxidant, but vitamin C plays an important role in conjunction with vitamin E in scavenging oxygen radicals and in protecting cell membranes (Niki et al., 1991).

1.6.3. DNA Damage

DNA is the cell’s genetic material, and any permanent damage to the DNA can result in changes (i.e., mutations) in the proteins encoded in the DNA, which may lead to malfunctions or complete inactivation of the affected proteins. Thus it is essential for the viability of individual cells or even the entire organism that the DNA remain intact. The building blocks of DNA molecules are called nucleotides; they consist of a sugar component and an organic base. Each DNA molecule consists of two strands of nucleotides held together by weak chemical bonds. Changes in the nucleotides in one strand can result in mismatches with the nucleotides in the other strand, yielding subsequent mutations. ROS are a major source of DNA damage, causing strand breaks, removal of nucleotides, and a variety of modifications of the organic bases of the nucleotides. Although cells have developed repair mechanisms to correct naturally occurring changes in the DNA, additional or excessive changes caused by ROS or other agents can lead to permanent changes or damage to the DNA, with potentially detrimental effects for the cell (Halliwell, 1999).

1.6.4. Diseases Involving Excessive ROS Levels

In addition to contributing to the development of ALD, ROS have been implicated in many other major diseases that plague humans. A partial listing of these conditions (Knight 1998; Kehrer 1993) includes:

- The toxic effects of $O_2$ itself, such as the oxidation of lipids and proteins, generation of mutations in the DNA, and destruction of cell membranes.
Cardiovascular diseases.
Atherosclerosis.
Various types of cancer.
Diabetes.
Neurodegenerative diseases, including Parkinson’s disease and Alzheimer’s disease.
Toxicity of heavy metals (e.g., iron).
Radiation injury.
Vitamin deficiency.
Toxicity of certain medications.
Inflammation, such as the destruction of joints, the synovial fluid that lubricates joints and one of its components (i.e., hyaluronic acid), as well as activation of inflammation–promoting signalling molecules called cytokines.
Toxic effects of tobacco smoke.
Emphysema.
Cataracts.

Finally, increasing evidence suggests that aging may be a consequence of the normal, longterm exposure to ROS and the accumulation of oxidized, damaged molecules within the cell—a process that could be likened to a lifetime of “rusting away.”

1.6.5. Systems Producing ROS

The major source of ROS production in the cell is the mitochondrial respiratory chain, which utilizes approximately 80 to 90 percent of the O₂ a person consumes. Thus, even though only a small percentage of that oxygen is converted to ROS, the mitochondrial respiratory chain in all cells generates most of the ROS produced in the body.

Another major source of ROS, especially in the liver, is a group of enzymes called the cytochrome P450 mixed–function oxidases. Many different variants of these iron–containing enzymes exist, some of which are responsible for removing or detoxifying a variety of compounds present in our environment and ingested (e.g., foods or drugs), including alcohol. Some cytochrome P450 enzymes also are important for metabolizing substances that naturally occur in the body, such as fatty acids,
cholesterol, steroids, or bile acids. The biochemical reactions spurred (i.e., catalyzed) by the cytochrome P450 molecules use molecular oxygen, and during these reactions small amounts of ROS are generated. The extent of ROS generation may vary considerably depending on the compound to be degraded and on the cytochrome P450 molecule involved. One type of cytochrome molecule that is especially active in producing ROS is known as CYP2E1. This enzyme is of particular interest when investigating alcohol–induced oxidative stress because its activity increases after heavy alcohol exposure and because CYP2E1 itself also metabolizes alcohol (Lieber 1997). Actually, CYP2E1 is also invariably elevated in the liver of patients with NASH because fatty acids (which increase in obesity) and ketones (which increase in diabetes) are also substrates for CYP2E1; their excess up-regulates CYP2E1. Although the pathogenesis of NAFLD and NASH has not yet been fully elucidated, a popular mechanism is the “Two Hit” theory, the first hit being the accumulation, by several causes (such as obesity), of fatty acids in the liver. The second hit is the peroxidation of these fatty acids because of the oxidative stress produced by different factors, such as CYP2E1 induction (Weltman et al., 1998).

ROS also are produced by a variety of oxidative enzymes present in cells, such as xanthine oxidase. Under normal physiological conditions, xanthine oxidase acts as a dehydrogenase—that is, it removes hydrogen from xanthine or hypoxanthine and attaches it to NAD, thereby generating NADH. However, under certain conditions, such as the disruption of blood flow to a tissue, xanthine dehydrogenase is converted to a ROS–producing oxidase form. Alcohol consumption also may promote the conversion of xanthine dehydrogenase to xanthine oxidase (Sultatos 1988), which can generate ROS, thereby enhancing oxidative stress.

Other sources of ROS in the body are two types of immune cells called macrophages and neutrophils, which help defend the body against invading microorganisms. In this case, however, ROS production is beneficial and even essential to the organism because it plays a central role in destroying foreign pathogens (Rosen et al. 1995). Macrophages and neutrophils contain a group of enzymes called the NADPH oxidase complex, which, when activated, generates superoxide radicals and hydrogen peroxide. Hydrogen peroxide then interacts with chloride ions present in the cells to produce hypochlorite (the active ingredient in bleach), which in turn destroys the pathogen. The NADPH oxidase complex and the resulting ROS production are
critical to the body’s defense against all kinds of diseases, as is evident in patients with a condition called chronic granulomatous disease, in which ROS production by the NADPH oxidase complex is drastically reduced. Patients with this condition are highly sensitive to infections and usually die at an early age.

Besides the ROS generation that occurs naturally in the body, humans are constantly exposed to environmental free radicals, including ROS, in the form of radiation, UV light, smog, tobacco smoke, and certain compounds referred to as redox cycling agents, which include some pesticides, but also certain medications used for cancer treatment. The toxicity of these medications against tumour cells (as well as normal body cells) results from the fact that the compounds are modified by cellular enzymes to an unstable intermediate, which then reacts with molecular oxygen to produce the original product plus a superoxide radical. Thus, a vicious cycle of chemical reactions involving these compounds continually produces ROS.

**1.6.6. Oxidative stress in ALD**

Alcohol induced oxidative stress may play a significant role in the development of ALD. Many processes and factors are involved in causing alcohol-induced oxidative stress (Defeng et al., 2003) including:

- Changes in the NAD⁺/NADH ratio in the cell as a result of alcohol metabolism. Alcohol is metabolized in two steps. First, the enzyme alcohol dehydrogenase converts alcohol to acetaldehyde, a toxic and reactive molecule. Next, the enzyme aldehyde dehydrogenase converts the acetaldehyde to acetate. Each of these reactions leads to formation of one molecule of NADH, thereby providing more starting material and thus enhanced activity of the respiratory chain, including heightened O₂ use and ROS formation.

- Production of acetaldehyde during alcohol metabolism, which through its interactions with proteins and lipids also can lead to radical formation and cell damage.

- Damage to the mitochondria resulting in decreased ATP production.

- Effects on cell structure (e.g., the membranes) and function caused by alcohol’s interactions with either membrane components (i.e., phosphate–containing lipids [phospholipids]) or enzymes and other protein components of the cells.
 Alcohol–induced oxygen deficiency (i.e., hypoxia) in tissues, especially in certain areas of the liver lobules (i.e., the pericentral region), where extra oxygen is required to metabolize the alcohol.

 Alcohol’s effects on the immune system, which lead to altered production of certain signalling molecules called cytokines, which in turn lead to the activation of an array of biochemical processes.

 Alcohol–induced increase in the ability of the bacterial molecule endotoxin to enter the bloodstream and liver, where it can activate certain immune cells.

 Alcohol–induced increases in the activity of the enzyme cytochrome P450 2E1 (CYP2E1), which metabolizes alcohol and other molecules and generates ROS in the process.

 Alcohol–induced increases in the levels of free iron in the cell (i.e., iron that is not bound to various proteins), which can promote ROS generation.

 Effects on antioxidant enzymes and chemicals, particularly a molecule called glutathione (GSH).

 Biochemical reactions generating an alcohol–derived radical (i.e., the 1–hydroxyethyl radical).

 Conversion of the enzyme xanthine dehydrogenase into a form called xanthine oxidase, which can generate ROS.

 Many of these processes operate concurrently, and it is likely that several, indeed many, systems contribute to the ability of alcohol to induce a state of oxidative stress.

 1.7. Protection Against ROS Toxicity

 Because ROS production is a naturally occurring process, a variety of enzymatic and nonenzymatic mechanisms have evolved to protect cells against ROS. At least some of these mechanisms are impaired after long–term alcohol consumption and may therefore contribute to damage to the liver and other organs.
1.7.1. Antioxidants

An antioxidant is a molecule capable of slowing or preventing the oxidation of other molecules. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidising agent. Oxidation reactions can produce free radicals, which start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates. Hence, antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. Consequently, organisms contain a complex network of antioxidant metabolites and enzymes that work together to prevent oxidative damage to cellular components such as DNA, protein and lipids (Sies H, 1997). Antioxidants are widely used as ingredients in dietary supplements in the hope of maintaining health and preventing diseases such as cancer and coronary heart disease (Bjelakovic et al., 2007). Antioxidants are classified into two broad divisions depending on whether they are soluble in water (hydrophilic) or in lipids (hydrophobic). In general, water-soluble antioxidants react with oxidants in the cell cytoplasm and the blood plasma, while lipid-soluble antioxidants protect cell membranes from lipid peroxidation (Sies H, 1997). These compounds may be synthesized in the body or obtained from the diet. The action of one antioxidant may therefore depend on the proper function of other members of the antioxidant system.
Vegetables contain a great quantity of non-nutritional antioxidants, such as flavonoids, flavones and total phenolic contents (Wang H, 1996). In general, antioxidant systems either prevent these reactive species from being formed, or remove them before they damage vital components of the cell. Fruits, vegetables, grains, tea, wine and some kinds of spices are natural sources of antioxidants. Dietary antioxidants serve as one of the source of protection that the body leads to protect against damaging effects of reactive species (Davies K, 1995).

The requisite characteristics for effective antioxidant molecules include a number of structural features. (i) The presence of hydrogen/ electron-donating substituents with appropriate reduction potentials. (ii) The ability to delocalize the resulting radical (Bors et al., 1990), whether a phenoxy radical such as those derived from α-tocopherol or butylated hydroxytoluene, a aryloxyl radical such as those derived from flavanoids, a polyunsaturated hydrocarbon chain radical such as β-carotene, or a thyl radical such as dihydrolipoic acid.(iii) The transition metal-chelating potential (Paganga et al., 1996) dependent on the nature of the functional groups and their arrangement within the molecule.

Under normal circumstances, cells are able to defend themselves against ROS damage with enzymes such as superoxide dismutases, catalases, lactoperoxidases, glutathione peroxidases and peroxiredoxins. Small molecule antioxidants such as ascorbic acid (vitamin C), tocopherol (vitamin E), and glutathione also play important roles as cellular antioxidants. In similar manner, polyphenol antioxidants assist in preventing ROS damage by scavenging free radicals.

1.7.2. Enzymatic Antioxidants

Enzymes involved in the elimination of ROS include superoxide dismutases (SODs), catalase, and glutathione peroxidise.

1.7.2.1. Superoxide dismutase

SODs catalyze the rapid removal of superoxide radicals. In mammals there are several types of SODs, which differ with respect to their location in the cells and the metal ions they require for their function. For example, a copper–zinc SOD is present in the fluid filling the cell (i.e., the cytosol) and in the space between the two membranes surrounding the mitochondria. Furthermore, a manganese–containing SOD
is present in the mitochondrial interior (i.e., matrix). Both of these enzymes are critical for prevention of ROS–induced toxicity (Fridovich 1997). The effects of chronic alcohol exposure on the cellular content or activity of SODs are controversial, with reports of increases, no changes, or decreases, depending on the model, diet, amount, and time of alcohol feeding. Studies employing a commonly used model in which alcohol is administered directly into the stomach of laboratory animals (i.e., the intragastric infusion model, used most commonly with rats and mice) found decreases in SOD activity in the liver (Polavarapu et al. 1998).

Superoxide dismutase is a class of closely related enzymes that catalyze the breakdown of superoxide anion into oxygen and hydrogen peroxide (Zelko et al., 2002). SOD enzymes are present in almost all aerobic cells and in extracellular fluids (Nozik et al., 2005). SOD enzymes contain metal ion cofactors and present in cytosol and mitochondria (Bennister et al., 1987).

Superoxide dismutases (SOD) are a class of enzymes that catalyze the dismutation of superoxide into oxygen and hydrogen peroxide. As such, they are an important antioxidant defense in nearly all cells exposed to oxygen. In mammals and most chordates, three forms of superoxide dismutase are present. SOD1 is located in the cytoplasm, SOD2 in the mitochondria and SOD3 is extracellular. The first is a dimer (consists of two units), while the others are tetramers (four subunits). SOD1 and SOD3 contain copper and zinc, while SOD2 has manganese in its reactive centre.

The SOD-catalysed dismutation of superoxide may be written with the following half-reactions:

\[
\begin{align*}
M^{(n+1)+} - \text{SOD} + O_2^- & \rightarrow M^{n+} - \text{SOD} + O_2 \\
M^{n+} - \text{SOD} + O_2^- + 2H^+ & \rightarrow M^{(n+1)+} - \text{SOD} + H_2O_2.
\end{align*}
\]

Where M = Cu (n=1); Mn (n=2); Fe (n=2); Ni (n=2).

In this reaction the oxidation state of the metal cation oscillates between n and n+1.

1.7.2.2. Catalase

Catalase and the glutathione peroxidase system both help to remove hydrogen peroxide. Catalase is an iron–containing enzyme found primarily in the small
membrane–enclosed cell components called peroxisomes (Del Rio et al., 1992). It serves to detoxify hydrogen peroxide and various other molecules. One way that catalase eliminates hydrogen peroxide is by catalyzing a reaction between two hydrogen peroxide molecules, resulting in the formation of water and O₂. In addition, catalase can promote the interaction of hydrogen peroxide with compounds that can serve as hydrogen donors so that the hydrogen peroxide can be converted to one molecule of water, and the reduced donor becomes oxidized (a process sometimes called the peroxidatic activity of catalase). Compounds that can provide these hydrogen atoms include beverage alcohol (i.e., ethanol) and methanol. Catalase catalyzes the conversion of hydrogen peroxide to water and oxygen using either iron or manganese as cofactor (Chelikani et al., 2004). Catalase is an unusual enzyme since, although hydrogen peroxide is its only substrate, it follows a ping-pong mechanism. Here, its cofactor is oxidised by one molecule of hydrogen peroxide and then regenerated by transferring the bound oxygen to a second molecule of substrate (Hiner et al., 2002).

\[
2 \text{H}_2\text{O}_2 \rightarrow 2 \text{H}_2\text{O} + \text{O}_2 \quad \text{(catalase)}
\]

Catalase, which is concentrated in peroxisomes located next to mitochondria, reacts with the hydrogen peroxide to catalyze the formation of water and oxygen. Glutathione peroxidase reduces hydrogen peroxide by transferring the energy of the reactive peroxides to a very small sulfur-containing protein called glutathione. The selenium contained in these enzymes acts as the reactive center, carrying reactive electrons from the peroxide to the glutathione. Peroxiredoxins also degrade H₂O₂, within the mitochondria, cytosol, and nucleus.

1.7.2.3. Glutathione peroxidase

Glutathione peroxidise is an enzyme containing four selenium cofactors that catalyzes the breakdown of hydrogen peroxide and organic hydroperoxides. These are atleast four different glutathione peroxidise isoenzymes in animals. These enzymes are present at particularly high levels in the liver and also serve in detoxification metabolism (Hayes et al., 2005). The glutathione peroxidase system consists of several components, including the enzymes glutathione peroxidase and glutathione reductase and the cofactors glutathione (GSH) and reduced nicotinamide adenosine dinucleotide phosphate (NADPH). Glutathione peroxidase contains an amino acid that is modified
by addition of a molecule of the metal selenium; therefore, low amounts of selenium are critical for the body’s antioxidant defense.) Together, these molecules effectively remove hydrogen peroxide. GSH, which consists of three amino acids, is an essential component of this system and serves as a cofactor for an enzyme called glutathione transferase, which helps remove certain drugs and chemicals as well as other reactive molecules from the cells. Moreover, GSH can interact directly with certain ROS (e.g., the hydroxyl radical) to detoxify them, as well as performing other critical activities in the cell.

Because of all its functions, GSH is probably the most important antioxidant present in cells. Therefore, enzymes that help generate GSH are critical to the body’s ability to protect itself against oxidative stress. Alcohol has been shown to deplete GSH levels, particularly in the mitochondria, which normally are characterized by high levels of GSH needed to eliminate the ROS generated during activity of the respiratory chain.

Mitochondria cannot synthesize GSH but import it from the cytosol using a carrier protein embedded in the membrane surrounding the mitochondria. Alcohol appears to interfere with the function of this carrier protein, thereby leading to the depletion of mitochondrial GSH (Fernandez–Checa et al. 1997). NADPH is involved in a much more diverse range of reactions in the cell than GSH. Nevertheless, because of its role in the glutathione peroxidase system, NADPH or the enzymes that generate this compound are sometimes considered antioxidants.

### 1.7.3. Non-Enzymatic antioxidants

The non-enzymatic antioxidants include vitamin A, vitamin C, vitamin E, carotenoids, lycopene, reduced glutathione and flavonoids.

Vitamin C is a water-soluble vitamin. Like vitamin E, it is an effective antioxidant. Vitamin C can protect essential substances in the body such as proteins, lipids, carbohydrates and DNA and RNA from damage by free radicals. Vitamin C works in tandem with vitamin E and is able to regenerate vitamin E when vitamin E is used as an antioxidant (Ball, 2004). Vitamin C is an electron donor and therefore a reducing agent. All known physiological and biochemical actions of vitamin C are due to its action as an electron donor. Ascorbic acid donates two electrons from a double
bond between the second and third carbons of the 6-carbon molecule. Vitamin C is called an antioxidant because, by donating its electrons, it prevents other compounds from being oxidized. However, by the very nature of this reaction, vitamin C itself is oxidized in the process (Bielski et al., 1975).

It is noteworthy that when vitamin C donates electrons, they are lost sequentially. The species formed after the loss of one electron is a free radical, semidehydroascorbic acid or ascorbyl radical. As compared to other free radicals (a species with an unpaired electron), ascorbyl radical is relatively stable with a half-life of 10-15 seconds and is fairly unreactive. This property explains why ascorbate may be a preferred antioxidant. In simple terms, a reactive and possibly harmful free radical can interact with ascorbate. The reactive free radical is reduced, and the ascorbyl radical formed in its place is less reactive. Reduction of a reactive free radical with formation of a less reactive compound is sometimes called free radical scavenging or quenching. Ascorbate is therefore a good free radical scavenger due to its chemical properties (Buettner et al., 1993).

Vitamin E is a major antioxidant found in the lipid phase of membranes and, like other chemically related molecules, acts as a powerful terminator of lipid peroxidation. During the reaction between vitamin E and a lipid radical, the vitamin E radical is formed, from which vitamin E can be regenerated in a reaction involving GSH and ascorbate. Alcohol also appears to interfere with the body’s normal vitamin E content because patients with ALD commonly exhibit reduced vitamin E levels (Nanji and Hiller–Sturmholfer 1997).

Vitamin E is a potent peroxyl radical scavenger (Burton et al., 1986) and can protect polyunsaturated fatty acids (PUFA) within phospholipids of biological membranes (Burton et al., 1983) and in plasma lipoproteins (Jialal et al., 1995). When vitamin E reacts with a peroxyl radical, it forms a tocopheroxyl radical. In this regard, α-tocotrienol is a more potent antioxidant than α-tocopherol . The higher antioxidant potency of α-tocotrienol is due to the combined effects of 1) its higher recycling efficiency from chromanoxyl radical, 2) its more uniform distribution in membrane bilayers, and 3) its stronger disordering of membrane lipids allowing interaction of the chromanol nucleus with lipid radicals (Serbinova et al., 1991).
Carotenoids are natural pigments which are synthesized by plants and are responsible for the bright colours of various fruits and vegetables. There are several dozen carotenoids in the foods that we eat, and most of the carotenoids have antioxidant activity. Antioxidants (including carotenoids) have been studied for their ability to prevent chronic disease. Beta-carotene and other carotenoids have antioxidant properties in vitro and in animal models. Carotenoids are also able to inhibit free radical reactions (Rice-Evans et al., 1997). The antioxidant actions of carotenoids are based on their singlet oxygen quenching properties and their ability to trap peroxyl radicals. The prevention of lipid peroxidation by carotenoids has been suggested to be mainly via singlet oxygen quenching (Stahl et al., 1996). At low concentrations and at low partial pressures of oxygen, β-carotene, was found to inhibit the oxidation of model compounds initiated by peroxy radicals (Kennedy and Liebler, 1991). Other free radicals such as nitrogen dioxide, thyl, and sulphonyl radicals are also scavenged rapidly by β-carotene (Everett et al., 1997). This antioxidant activity of β-carotene, which is shared by other carotenoids as well (Lim et al., 1992), may contribute to the protection of membranes from lipid peroxidation. Mixture of carotenoids associated with other antioxidants (e.g. vitamin E) can increase their activity against free radicals (Sergio et al., 1999). Carotenoids partially or completely protect intact cells (e.g. human liver cell line HepG2) against oxidant-induced lipid peroxidation, and the protective effect is independent of provitamin A activity (Martin et al., 1996).

Lycopene, a member of the carotenoids family is found in human plasma and tissues (Leticia et al., 2003). Lycopene was considered to be an antioxidant that can donate electrons to quench and neutralize free radical oxygen molecule that are known to accelerate ageing and damage cells. Lycopene gives tomatoes and other fruits and vegetables a red colour. The synthesis of lycopene has also been found in some photosynthetic organisms like algae, some types of fungi and some bacteria whereas in animals and in human they are incorporated from the diet (Kaia et al., 2004). Increased levels of lycopene have been associated with prevention of prostate cancer and coronary heart disease (Gerster et al., 1997).

It is widely distributed in nature and exists in reduced or oxidised states. The reversible oxidation-reduction of glutathione is important for many of its biological function. Glutathione protects many –SH group containing enzymes from oxidation of their –SH group. Glutathione has antioxidant properties since the thiol group in its
cysteine moiety is a reducing agent and can be reversibly oxidised and reduced. In cells, glutathione is maintained in reduced form by the enzyme glutathione reductase (Meister, 1994). Due to its high concentration and its central role in maintaining the cell’s redox state, glutathione is one of the most important cellular antioxidants (Meister, 1994). In some organism glutathione is replaced by other thiols, such as by mycothiol in actinomycetes, or by trypanothione in the kinetoplastids (Fahey et al., 2001). Glutathione, an important water-soluble antioxidant, is synthesized from the amino acids glycine, glutamate, and cysteine. Glutathione directly quenches ROS such as lipid peroxides, and also plays a major role in xenobiotic metabolism. Exposure of the liver to xenobiotic substances induces oxidative reactions through the upregulation of detoxification enzymes, i.e., cytochrome P-450 mixed-function oxidase. When an individual is exposed to high levels of xenobiotics, more glutathione is utilized for conjugation (a key step in the body’s detoxification process) making it less available to serve as an antioxidant. Research suggests that glutathione and vitamin C work interactively to quench free radicals and that they have a sparing effect upon each other (Jacob, 1995).

Flavonoids, a subclass of polyphenols, are a group of phytochemicals that are among the most potent and abundant antioxidants in our diet, and are a class of water soluble plant pigments. Flavonoids which are present in most plants, concentrating in seeds, fruit skin or peel, bark and flowers. A great number of plant medicines contain flavonoids, which have been reported to possess antioxidant, anti ulcer, anti ageing, antibacterial, anti-inflammatory, anti-allergic, anti-mutagenic, anti-viral, anti fungal, anti diabetic, anti hepatotoxic, anti-neoplastic, anti tumour, and vasodilatory actions (Shohaib et al., 2011). Flavonoids have been shown in number of studies to be potent antioxidants, capable of scavenging hydroxyl radicals, superoxide anions and lipid peroxy radicals (Alan et al., 1996).

The liver is subject to acute and potentially lethal injury by several substances including phalloidin (the toxic constituent of the mushroom, Amanita phalloides), CCl4, galactosamine, ethanol, and other compounds. Flavonoids have also been found to possess hepatoprotective activity. In a study carried out to investigate the flavonoid derivatives silymarin, apigenin, quercetin, and naringenin, as putative therapeutic agents against microcrystin LR-induced hepatotoxicity, silymarin was found to be the most effective one (Carlo et al., 1993). The flavonoid, rutin and venoruton, showed regenerative and hepatoprotective effects in experimental cirrhosis (Lorenz et al., 1994).
Excess levels of ROS and the resulting oxidative stress have been implicated in a variety of human diseases. Many studies have demonstrated that alcohol increases lipid peroxidation as well as the modification of proteins; however, it is not always clear if these changes are the causes rather than consequences of alcohol–induced tissue injury. Nevertheless, numerous investigations have found that administering antioxidants, agents that reduce the levels of free iron, or agents that replenish GSH levels can prevent or ameliorate the toxic actions of alcohol. For example, in the intragastric infusion model, the antioxidant vitamin E; the chemical epsilon, which mimics the actions of glutathione peroxidase; the copper–zinc or manganese SODs; or a GSH precursor-all prevented ALD (Limuro et al. 2000; Kono et al. 2001; Wheeler et al. 2001).

Accordingly, the health benefits of administering antioxidants such as vitamins E and C or other compounds are the subject of much current research, and clinical trials employing antioxidants in the treatment of various conditions are under way. For example, some therapeutic interventions with antioxidants have shown success or promise in the treatment of Parkinson’s disease and in reducing the toxicity of the cancer medication adriamycin.

Not all instances of ROS production are detrimental to the organism, however. One beneficial effect is the production of ROS by certain immune cells in order to destroy invading foreign organisms (Rosen et al. 1995). Furthermore, recent evidence suggests that ROS, especially hydrogen peroxide, may be important in signal transduction mechanisms in cells and thus may be an integral component of cellular physiology and metabolism (Lander 1997).

In addition to these studies conducted with intact animals (i.e., in vivo), studies with liver cells (i.e., hepatocytes) grown in culture also showed that alcohol can produce oxidative stress and hepatocyte toxicity. Studies with hepatocytes isolated from control rats or from rats that continuously had been fed alcohol indicated that alcohol metabolism via the enzyme alcohol dehydrogenase results in increased ROS production, hepatocyte injury, and a type of cell death known as apoptosis. Moreover, all of these reactions could be blocked by the administration of antioxidants (Adachi and Ishii 2002; Bailey and Cunningham 2002). Finally, studies using an established hepatocyte cell line that contains the alcohol–metabolizing and ROS–producing
enzyme CYP2E1 demonstrated that adding alcohol, polyunsaturated fatty acids, or iron, as well as reducing GSH, resulted in cell toxicity, increased oxidative stress, and mitochondrial damage (Wu and Cederbaum 1999). Furthermore, all of these reactions could be prevented by administering antioxidants. Taken together, these findings indicate that alcohol–induced oxidative stress is a pivotal factor in the development of ALD.

1.8.1. Ayurvedic concept

The Ayurvedic concept appeared and developed between 2500 and 500 BC in India. The literal meaning of Ayurveda is “science of life,” because ancient Indian system of health care focused views of man and his illness. It is pointed out that the positive health means metabolically well-balanced human beings. According to Ayurveda, the disease evolves from the body due to external factors. It has a vast literature in Sanskrit covering all aspect of diseases, pharmacy and therapeutics. The practice of Ayurveda therapeutics consisted of 8 sections divided into 180 chapters and listed 314 plants, which are used as medicines in India (Subhose, 2005). The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments (Ballabh et al., 2007). Many Westerners have long regarded the Indian systems of medicine as a rich source of knowledge (Subhose, 2005). In India, around 20,000 medicinal plants have been recorded (Dev,1997), however traditional communities are using only 7,000 - 7,500 plants for curing different diseases (Perumalsamy et al., 1998, Perumalsamy et al., 2000, Kamboj, 2000).

The medicinal plants are listed in various indigenous systems such as Siddha (600), Ayurveda (700) and Amchi (600), Unani (700), Allopathy which 30 plant species for ailments (Rabe et al., 1997). The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve the traditional heritage but also to rationalize the use of traditional medicines in the health care (Aravind et al., 2008). Even today, majorities of the medicines are prepared from the plant and animal products, minerals and metals etc. Major pharmaceutical industries depend on the plant products for the preparation of Ayurvedic medicines. In the present context, the Ayurvedic system of medicine is widely accepted and practiced not only in the Indian Peninsula but also in the developed countries such as Europe, United States and Japan. Plant derived medicines
have been the first line of defense in maintaining health and combating diseases (John, 1984, Veale, 1992). In the last century, roughly 121 pharmaceutical products have been discovered based on the information obtained from the traditional healers (Anesini et al., 1993).

Chemical principles from natural sources have become much simpler and have contributed significantly to the development of new drugs from medicinal plants (Cox,1990, Cox,1994). Biologically active compounds from natural sources have always been of great interest to scientists working on infectious diseases. Research to find out scientific evidence for claims of plants used for Indian Ayurvedic system of medicine has been intensified. Detailed research on the chemistry and pharmacology of products of plant origin are much essential and this may eventually lead to the discovery of medicine that can be used in the treatment of several diseases (Dev, 1997). Moreover, these local Ayurvedic preparations are scientifically evaluated and disseminated properly, our indigenous population can be given better access to efficacious drug treatment and improved health status (Manandhar, 1985, Manandhar, 1987). However, over commercial exploitation of these plant (herbal) products frequently degradation of natural resources are reported to be major threats to medicinal plants in India. The aim of the present study is to understand the knowledge of plants used for Ayurvedic preparations, can be extended for future scientific investigation near future.

1.8.2. Plants in traditional medicines

Four thousand years ago, the medical knowledge of the Indian subcontinent was termed as Ayurveda. Ayurveda remains an important system of medicine and drug therapy in India. Plant alkaloids are the primary active ingredients of Ayurvedic drugs. Today the pharmacologically active ingredients of many Ayurvedic medicines are being identified and their usefulness in drug therapy being determined. As mentioned in the introduction only a certain percentage of plants are used in traditional medicines. It is roughly estimated that of the discovered 17,000 species, nearly 3,000 species are used in medicinal field (Nayar, 1987). The therapeutic action of important medicinal plants and its parts used. The scientific study of traditional medicines, derivation of drugs through bioprospecting and systematic conservation of the concerned medicinal plants are thus of great importance. Green plants synthesise and
preserve a variety of biochemical products, many of which are extractable and used as chemical feed stocks or as raw material for various scientific investigations. Many secondary metabolites of plant are commercially important and find use in a number of pharmaceutical compounds. According to WHO, around 21,000 plant species have the potential for being used as medicinal plants. About 5000 species have been studied. There are at least 121 major plant drugs of known structure, but none of them is currently produced through synthetic means. For developing phytomedicines as a major area of concern, it would be essential to adopt a holistic interdisciplinary approach, have a scientific basis of the understanding of the plant systems, new innovations and their conservation for utilisation in future on a sustainable basis (Sharma, 1997).

1.8.3. Medicinal plants frequently used in Ayurvedic formulation

Ayurveda, whose history goes back to 5000 BC., is one of the ancient health care systems. The Ayurveda was developed through daily life experiences with the mutual relationship between mankind and nature. The ancient text of Ayurveda reports more than 2000 plant species for their therapeutic potentials. Besides Ayurveda, other traditional and folklore systems of health care were developed in the different time periods in Indian subcontinent, where more than 7500 plant species were used. According to a WHO estimate, about 80% of the world population relies on traditional systems of medicines for primary health care, where plants form the dominant component over other natural resources (Ram, 1997). Ayurveda, pancavidha kasayakalpana are the two basic pharmaceutical preparations, from which all the other preparations are formulated. Sarangdhara mentioned detailed information about various formulations with respect to their methods of preparation as well as basic standards and are documented in Sarangdhara Samhita (Mukherjee et al., 2006). The different drug is prepared by percentage dry weight of the plant parts for the Ayurvedic formulations.

The traditional knowledge with its holistic system approach supported by experimental base can serve as an innovative and powerful discovery engine for newer, safer and affordable medicines. These plant species mentioned in the ancient texts of Ayurvedic and other Indian systems of medicines may be explored with the modern scientific approaches for better leads in the health care. Hence, the
The present review is focused on an overall outline of plant used in Ayurvedic drug scenario and its future prospects for the further scientific investigations. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on Ayurvedic medicinal plants. Several preclinical and clinical studies have examined cytotoxic, immunomodulatory and immunoadjuvant potential of Ayurvedic medicines. The development of these traditional systems of medicines with the perspectives of safety, efficacy and quality will help not only to preserve this traditional heritage but also to rationalize the use of natural products in the health care. Thus, we can easily identify rare and extinct plants for the conservation and preserved the traditional heritage of the traditional practitioners.

The past decade has witnessed a tremendous resurgence in the interest and use of medicinal plant products, especially in North America. Surveys of plant medicinal usage by the American public have shown an increase from just about 3% of the population in 1991 to over 37% in 1998 (Brevoort, 1998). The North American market for sales of plant medicinals has climbed to about $3 billion/year (Glaser, 1999). Once the domain of health-food and specialty stores, phytomedicines have clearly re-emerged into the mainstream as evidenced by their availability for sale at a wide range of retail outlets, the extent of their advertisement in the popular media, and the recent entrance of several major pharmaceutical companies into the business of producing phytomedicinal products (Brevoort, 1998; Glaser, 1999). No doubt a major contributing factor to this great increase in phytomedicinal use in the United States has been the passing of federal legislation in 1994 (Dietary Supplement Health and Education Act or “DSHEA”) that facilitated the production and marketing of phytomedicinal products (Brevoort, 1998).

The past decade has also witnessed intense interest in “Nutraceuticals” (or “Functional foods”) in which phytochemical constituents can have long-term health promoting or medicinal qualities. Although the distinction between medicinal plants and nutraceuticals can sometimes be vague, a primary characteristic of the latter is that nutraceuticals have a nutritional role in the diet and the benefits to health may arise from long-term use as foods (i.e. chemoprevention) (Korver, 1998). In contrast, many medicinal plants exert specific medicinal actions without
serving a nutritional role in the human diet and may be used in response to specific health problems over short- or long-term intervals.

In recent years, secondary plant metabolites (Phytochemicals), previously with unknown pharmacological activities, have been extensively investigated as a source of medicinal agents. The World Health Organization has given guidelines to the member states to ensure about genuine use of plants and their parts before their use for human health (Krisharaju et al., 2000).

For many of the medicinal plants of current interest, a primary focus of research to date has been in the areas of phytochemistry, pharmacognosy, and horticulture. In the area of phytochemistry, medicinal plants have been characterized for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis. Research in the pharmacognosy of medicinal plants has also involved assays of bio-activity, identification of potential modes of action, and target sites for active phytomedicinal compounds. Horticultural research on medicinal plants has focused on developing the capacity for optimal growth in cultivation. This has been especially pertinent as many medicinal plants are still harvested in the wild, and conditions for growth in cultivation have not been optimized. Wild harvesting of medicinal plants can be problematic in terms of biodiversity loss, potential variation in medicinal plant quality, and occasionally, improper plant identification with potential tragic consequences.

1.8.4. Plant secondary metabolites

Phytochemicals, especially flavonoids and phenolic acids, are of current interest because of their important biological and pharmacological properties. The beneficial medicinal effects of plant materials typically result from the combinations of secondary products present in the plant. That the medicinal actions of plants are unique to particular plant species or groups is consistent with this concept as the combinations of secondary products in a particular plant are often taxonomically distinct (Wink, 1999). This is in contrast to primary products, such as carbohydrates, lipids, proteins, heme, chlorophyll, and nucleic acids, which are common to all plants and are involved in the primary metabolic processes of building and maintaining plant cells (Kaufman et al., 1999; Wink, 1999). Although plant secondary products have historically been defined as chemicals that do not appear to have a vital biochemical role in the process of
building and maintaining plant cells, recent research has shown a pivotal role of these chemicals in the ecophysiology of plants. Accordingly, secondary products have both a defensive role against herbivory, pathogen attack, and inter-plant competition and an attractant role toward beneficial organisms such as pollinators or symbionts (Kaufman et al., 1999; Wink and Schimmer, 1999). Plant secondary products also have protective actions in relation to abiotic stresses such as those associated with changes in temperature, water status, light levels, UV exposure, and mineral nutrients (Kaufman et al., 1999). Furthermore, recent work has indicated potential roles of secondary products at the cellular level as plant growth regulators, modulators of gene expression, and in signal transduction (Kaufman et al., 1999).

Although secondary products can have a variety of functions in plants, it is likely that their ecological function may have some potential medicinal effects for humans. For example, secondary products involved in plant defense through cytotoxicity toward microbial pathogens could prove useful as antimicrobial medicines in humans, if not too toxic. Likewise, secondary products involved in defense against herbivores through neurotoxin activity could have beneficial effects in humans (i.e. as antidepressants, sedatives, muscle relaxants, or anesthetics) through their action on the central nervous system. To promote the ecological survival of plants, structures of secondary products have evolved to interact with molecular targets affecting the cells, tissues, and physiological functions in competing microorganisms, plants, and animals (Wink and Schimmer, 1999). In this respect, some plant secondary products may exert their action by resembling endogenous metabolites, ligands, hormones, signal transduction molecules, or neurotransmitters and thus have beneficial medicinal effects on humans due to similarities in their potential target sites (e.g. central nervous system, endocrine system, etc.) (Kaufman et al., 1999). As noted by Wink (1999), the development of structural similarity between plant secondary products and the endogenous substances of other organisms could be termed “evolutionary molecular modeling.”

In contrast to synthetic pharmaceuticals based upon single chemicals, many phytomedicines exert their beneficial effects through the additive or synergistic action of several chemical compounds acting at single or multiple target sites associated with a physiological process. As pointed out by Tyler (1999), this synergistic or additive pharmacological effect can be beneficial by eliminating the problematic side effects
associated with the predominance of a single xenobiotic compound in the body. In this respect, Kaufman et al., (1999) extensively documented how synergistic interactions underlie the effectiveness of a number of phytomedicines. This theme of multiple chemicals acting in an additive or synergistic manner likely has its origin in the functional role of secondary products in promoting plant survival. For example, in the role of secondary products as defense chemicals, a mixture of chemicals having additive or synergistic effects at multiple target sites would not only ensure effectiveness against a wide range of herbivores or pathogens but would also decrease the chances of these organisms developing resistance or adaptive responses (Kaufman et al., 1999; Wink, 1999).

1.9.1. Molecular Docking study

The application of computational methods to study the formation of intermolecular complexes has been the subject of intensive research during the last decade. It is widely accepted that drug activity is obtained through the molecular binding of one molecule (the ligand) to the pocket of another, usually larger, molecule (the receptor), which is commonly a protein. In their binding conformations, the molecules exhibit geometric and chemical complementarity, both of which are essential for successful drug activity. The computational process of searching for a ligand that is able to fit both geometrically and energetically the binding site of a protein is called molecular docking.

In the field of molecular modelling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules using scoring functions. Docking is frequently used to predict the binding orientation of small molecule drug candidates to their protein targets in order to in turn predict the affinity and activity of the small molecule. Hence docking plays an important role in the rational design of drugs. Given the biological and pharmaceutical significance of molecular docking, considerable efforts have been directed towards improving the methods used to predict docking.
One key aspect of molecular modelling is calculating the energy of conformations and interactions. This energy can be calculated with a wide range of methods ranging from quantum mechanics to purely empirical energy functions. The accuracy of these functions is usually proportional to its computational expense and choosing the correct energy calculation method is highly dependent on the application. Computation times for different methods can range from a few milliseconds on a workstation to several days on a massively parallel supercomputer. In the context of docking, energy evaluations are usually carried out with the help of a scoring function and developing these is a major challenge facing structure based drug design (Vieth et al., 1998). Scoring functions are a critical part of the structure based drug design process.
No matter how efficient and accurate the geometric modeling of the binding process is, without good scoring functions it is impossible to obtain correct solutions. The two main characteristics of a good scoring function are selectivity and efficiency. Selectivity enables the function to distinguish between correctly and incorrectly docked Structures and efficiency enables the docking program to run in a reasonable amount of time.

A large number of current scoring functions are based on forcefields that were initially designed to simulate the function of proteins (Mackerell et al., 1998, Cornell, 1995). A forcefield is an empirical fit to the potential energy surface in which the protein exists and is obtained by establishing a model with a combination of bonded terms (bond distances, bond angles, torsional angles, etc.) and non-bonded terms (van der Waals and electrostatic). The relative contributions of these terms are adjusted for the different types of atoms in the simulated molecule by adjusting a series of parameters. Some scoring functions used in molecular docking have been adapted to include terms such as solvation and entropy (Morris, 1998). A separate approach is to use pure empirical scoring functions that are derived using multivariate regression methods of experimental data.

Ideally ligand docking to a protein could be simulated using Molecular Dynamics (MD). This has the advantage that not only it takes into account all the degrees of freedom available to the protein but also enables an explicit modeling of the solvent. Furthermore, accurate energy calculations can also be carried out using the free energy perturbation method. Unfortunately, modeling proteins using MD is computationally expensive, and the computational power necessary to simulate the full process of diffusion and ligand binding without any approximations will be out of our reach for many years to come. Recently Mangoni et al (1999) reported a modification to the standard MD protocol which reduces the computational time required for the docking simulation. The protocol consists of separating the center of mass motion of the ligand from its internal and rotational motions by coupling the different degrees of freedom to separate thermal baths. This optimization allows the ligand to sample the space surrounding the binding site faster while maintaining correct interactions with both protein and solvent. An alternative approach to model full protein flexibility is to generate an ensemble of rigid protein conformations that together represent the conformational diversity available to the protein. These conformations can later be docked to database of ligands using traditional rigid-protein/flexible-ligand methods.
There are several possible methods to generate the ensembles, but unfortunately their accuracy is proportional to the difficulty in obtaining them. The most accurate ensemble is the one determined exclusively from experimental data. An example is the case where several structures of protein/ligand complexes are determined using X-ray crystallography bound to different candidate drugs. Under these circumstances it is usually possible to observe alternative binding modes directly (Munshi, 2000).

Another less accurate option is to use the ensemble of structures that results from an experimental protein structure determination using the NMR (Nuclear Magnetic Resonance) technique. This docking methodology was first reported by Knegt et al. Finally, one can generate an ensemble using computational methods such as Monte Carlo (MC) or MD sampling. The accuracy of these alternatives is closely related to the accuracy of the force field used and is limited by the ability of these computational techniques to effectively sample the conformational space (Clarage et al., 1995). Docking to an ensemble of structures generated using MD was first reported by Pang and Kozikowski (Pang et al., 1994). A different representation for full protein flexibility is to divide the protein in tightly coupled domains whose constituent atoms move collectively as one. Hinges connect the domains and the motion of the protein is simulated similarly to an articulated robot. Required conformational changes inside domains can be handled using minimization. An application of this model to the docking problem was reported by Sandak et al., 1998.

1.10. Herbal option

The plants used in the present study are *Entada pursaetha*, *Toddalia aculeata*, and *Ziziphus mauritiana*. The seeds of *Entada pursaetha*, the stem of *Toddalia aculeata*, and the fruits of *Ziziphus mauritiana* were used in the formulation of Hippo-08.

**Plate 1:** Composition of Ayurvedic Formulation.

- Entada pursaetha
- Toddalia aculeata
- Ziziphus mauritiana
**Entada pursaetha**

*Entada pursaetha* (Elephant creeper) belonging to the family Fabaceae, is an endemic woody liane rarely distributed in the subtropical evergreen forests of Western Ghats of Karnataka, Tamil Nadu and Kerala and Eastern Ghats of Andhra Pradesh (Pullaiah et al., 1998). In the Indian system of medicine, the plant is called as Bidhanta (Sanskrit) and locally as Yaanaikazhachikaai. The seed kernel is a potential source of drug for various ailments such as cancer, liver disorders, dropsy, eye diseases, cuts, wounds, snakebite, respiratory problems, debility, tuberculosis and anasarca (Liu et al., 1972).

Conventionally, the plant is propagated only through seeds, which are largest (6 to 8 cm dia) among the angiosperms. The pod is 1.5 to 2 m long and 10-12 cm broad. The seed coat is very thick and hard. The dormancy period of seeds prolongs up to 5 years. This species is under severe threat due to destruction of tropical evergreen habitat and unscientific overexploitation of the plant parts like bark and seeds for medicinal purposes, which has resulted in the dwindling of population in the wild.

A phytoconstituent, entagenic acid was isolated from the seed kernel of *Entada pursaetha*, which was shown to possess antibacterial activity. Five new triterpenoid saponins, pursaethosides A-E (1-5), were isolated from the n-BuOH extract of the seed kernels of Entada pursaetha along with the known phaseoloidin (Tapondjou et al., 2005).

**Toddalia aculeata**

*Toddalia aculeata* is a thorny large shrub belongs to the family Rutaceae, always occur in forests near rivers or streams. It grows fairly well in clay soil. It has been used by traditional health practitioners in East Africa for management of diseases. The root bark is credited with diaphoretic, stomachic, antipyretic and antimalarial properties. Essential oils shows antimicrobial activity (Saxena and Sharma., 1999)

This woody liana can read a height of 10m in forests as it uses other trees for support. The corky stems are covered with knobby thorns and are yellow when cut, the attractive shiny trifoliolate leaves are light to dark green and are extremely aromatic, smelling of lemon when crushed. The fruits are covered in small, recurred thorns.

From the root bark of *Toddalia aculeata*, two alkaloids (Toddaline and Toddalinine) were isolated. Two more alkaloids were isolated from methanol extracts of leaves and stems of *Toddalia aculeata*, and the spectral studies including NMR was done (Subhash et al., 2006).
Ziziphus mauritiana

*Ziziphus mauritiana* is a tropical fruit tree species belonging to the family Rhamnaceae, originally native of India. The fruits are sweet, cooling, anodyne purgative, mucilaginous, pectoral, Styptic, aphrodisiac, invigorative, depurative, appetizer and tonic. It is much branched, thorny, deciduous tree with spreading crown, dark greyish black bark having irregular cracks and strong reddish hardwood. The leaves are elliptic and the apex toothed. The fruits are oblong or round, turning from yellow to orange and finally red, the fleshy pulp enclosing a hard stone.

The fruits are applied on cuts and ulcers; are employed in pulmonary ailments and fevers; and, mixed with salt and chili peppers, are given in indigestion and biliousness. The dried ripe fruit is a mild laxative. The seeds are sedative and are taken, sometimes with buttermilk, to halt nausea, vomiting, and abdominal pains in pregnancy. They check diarrhea, and are poulticed on wounds. Mixed with oil, they are rubbed on rheumatic areas. (Morton, 1987). The leaves are applied as poultices and are helpful in liver troubles, asthma and fever and, together with catechu, are administered when an astringent is needed, as on wounds. The bitter, astringent bark decoction is taken to halt diarrhea and dysentery and relieve gingivitis. The bark paste is applied on sores. The phenolic compounds like *p*-hydroxybenzoic acid, caffeic acid, ferulic acid and *p*-coumaric acid were identified in *Ziziphus mauritiana* fruit. Recently it has been proved that the seeds *Ziziphus mauritiana* has potent anticancer activity. The enhanced antioxidant status, decreased LPO and increased levels of GSH, CAT & SOD were also observed (Mishra et al., 2011)

<table>
<thead>
<tr>
<th>Table-1 Taxonomy of Selected Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kingdom</strong></td>
</tr>
<tr>
<td><strong>Division</strong></td>
</tr>
<tr>
<td><strong>Class</strong></td>
</tr>
<tr>
<td><strong>Subclass</strong></td>
</tr>
<tr>
<td><strong>Order</strong></td>
</tr>
<tr>
<td><strong>Family</strong></td>
</tr>
<tr>
<td><strong>Genus</strong></td>
</tr>
<tr>
<td><strong>Species</strong></td>
</tr>
</tbody>
</table>
OBJECTIVES

The study was deliberate to screen the secondary metabolites, enzymatic and non-enzymatic antioxidants effect on ethanolic extract of Hippo-08 with the following objectives.

1. Screening of Phytochemical and Biochemical constituents of Hippo-08 oral ayurvedic formulation.
   - Determination of secondary metabolites of Hippo-08. [Qualitative / Quantitative]
   - Screening and characterization of Hippo-08 by GC-MS and HPTLC analysis.

2. Access to the antioxidant property of Hippo-08 by in vitro assay
   i. Free radical scavenging assays:
      - 1, 1-diphenyl-2-picrylhydrazyl [DPPH] scavenging activity,
      - 2, 2’-azinobis-(3-ethyl benzothiazoline-6-sulphonic acid [ABTS] scavenging activity,
      - Superoxide radical scavenging activity,
      - Nitric oxide radical scavenging activity,
      - Hydrogen peroxide radical scavenging activity,
      - Hydroxyl radical scavenging activity,
      - Ferric reducing power assay
      - Determination of total antioxidant activity
   ii. Assay of Enzymatic antioxidants [SOD, CAT, GPx, GST and GR]
   iii. Assay of Non-enzymatic antioxidants [reduced glutathione, carotenoids, lycopene, Vitamin-A, Vitamin-C and Vitamin-E]

3. To ascertain the hepatoprotective effect and antioxidant activity of Hippo-08 by in vivo studies [animal experimentation]
   - Induction of alcoholic fatty liver in rats.
   - To assess the effect of Hippo-08 against alcoholic fatty liver. [AFL]
   - Comparison of the hepatoprotective effect induced by Hippo-08 and liv-52 [standard drug] against alcoholic fatty liver in rats.
i. **Acute and sub acute toxicity studies**
   - Quantification of the biochemical constituents [Glucose, Total protein, Albumin, Urea and Creatinine]
   - Study on Lipid profile [Total cholesterol / Triglycerides and Total Bilirubin]
   - Assay of Liver Marker Enzymes [AST, ALT and ALP]
   - Measurement of organs weights and body weight.
   - Assessment of Hematological parameters.
   - Histopathology of liver.

ii. **Chronic study**
   - Animal modeling and grouping
   - Measurement of Body weight and Liver weight
   - Assessment of Liver Marker Enzymes (AST, ALT and ALP)
   - Quantification of Glucose, Total Protein, Albumin, Urea, Creatinine Total Bilirubin
   - Lipid profile [Total Cholesterol, Tri glycerides, VLDL, LDL and HDL]
   - Assay of Enzymatic and Non-enzymatic antioxidants.
   - Histopathological study (liver).

4. **Molecular docking study with CYP2E1 and CYP3A4**

The study was categorized into five major chapters

CHAPTER I: Introduction
CHAPTER II: Phytochemical and Biochemical characterization of Hippo-08
CHAPTER III: Determination of Antioxidant property of *Hippo-08* by *In vitro* Assay.
CHAPTER IV: Evaluation of Hepatoprotective and Antioxidant property of *Hippo-08 – In vivo* studies (animal experimentation)
CHAPTER V: Molecular docking study.