Review of Literature
Alcohol is consumed to excess by large population all over the world and the number of alcohol consumers has been increasing alarmingly in developing countries including India (Nirmala and Jeyanthi 1987; Gronbaek et al. 1998). Excess in this sociological context refers to an amount sufficient to impair, social, economic and sexual functioning (Tavil and Cooksley 1983). Besides these, other problems such as neurological, gastrointestinal and cardiological disorders may develop in alcoholics. However, alcohol is widely included as one of the components in pharmaceutical preparations also due to its beneficiary effects as a drug, calorigenic and relaxant (Madan 1987; Hoek and Taraschi 1988; Christiansen et al. 1994). Chronic and excessive drinking not only disturbs healthy life organisation, it also affects many organs and metabolism in humans and experimental animals (Ramakrishnan et al. 1976; Reitz 1979; Topping et al. 1979; Preedy and Peters 1989; Hoek et al. 1992; Ponnappa et al. 1993; Kaur et al. 1994; Renis et al. 1996; Lands 1997; Gronbaek et al. 1998). Alcohol abuse and addiction are the leading causes of domestic violence and highway deaths (Narayana Reddy 1994; Bellen 1998). Under the influence of alcohol, one may turn into a scoundrel and criminal and forsake his home, family and his business. However, it will not be appropriate to say that any adult who occasionally drinks, is a candidate for personal disorganisation. There would not have been the problem of alcoholism had it been possible to restrict its use to moderation. Though some state governments have imposed prohibition on alcohol consumption, the implementation has not been effective because of various reasons. Reports clearly indicate that the incidence of alcoholism is on the increase.
In order to bring the necessary awareness among people regarding the consequences of alcoholism, an understanding of physiological mechanisms, molecular pathways, pathological phenomena and genetic pathways leading to abnormality is greatly warranted. Also this would pave the way for a better drug design and possibly help or even cure patients with the problems associated with alcoholism (Bellen 1998).

**Alcohol, its chemistry and pharmacology**

The term alcohol in common use refers to ethyl alcohol (C₂H₅OH). It is a transparent, colourless, volatile liquid having a characteristic spiritous odour with a burning taste. Alcohol beverages are a mixture of alcohol and water with small amounts of organic acids and esters. Alcohol content differs in different beverages as given below:

<table>
<thead>
<tr>
<th>Type</th>
<th>Alcohol Content</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rum, liquor</td>
<td>50 – 60%</td>
</tr>
<tr>
<td>Gin, arrack, whisky, brandy</td>
<td>40 – 45%</td>
</tr>
<tr>
<td>Port, sherry</td>
<td>20%</td>
</tr>
<tr>
<td>Wine</td>
<td>10 – 15%</td>
</tr>
<tr>
<td>Beers</td>
<td>4 – 8%</td>
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</tbody>
</table>

Arrack is a commonly used liquor by lower class Indian population. It is distilled from palm, rice, sugar or jaggery etc., and mixed with chloral hydrates and potassium bromide for getting greater kick (Narayana Reddy 1994).
Alcohol requires no digestion prior to absorption. Ethanol is a weakly charged molecule and moves easily through cell membranes, rapidly equilibrating between blood and tissues. Immediately after ingestion, alcohol is absorbed from stomach (20%) and through small intestine (80%). Studies also indicate that alcohol is readily absorbed from all parts of the digestive tract (mucous membrane, oesophagus, stomach, intestine, and colon (Franz 1983; Narayana Reddy 1994). Unlike other anaesthetics, alcohol is consumed in relatively large quantities for longer periods. Blood alcohol levels reach maximum concentration in few minutes after ingestion of alcohol beverage like whisky or beer and about a level of 20 mM ethanol in blood is indicative of intoxication (Hoek and Taraschi 1988). Carbonation hastens absorption, whereas food delays it. Alcohol from blood is cleared rapidly as little is lost through lungs by diffusion and its further passage to all other tissues through capillary net work with which it comes in contact till it reaches equilibrium between blood and tissues except adipose tissue (Narayana Reddy 1994). About 80% of alcohol absorbed is oxidised in the liver and remaining 10% is excreted in breath, urine, sweat, saliva, milk, tears and faeces.

Ethanol is a central nervous system depressant that decreases the activity of neurons, although some behavioural stimulation is observed at low blood levels. This drug has cross tolerance and shares a similar pattern of behavioural problems with other brain depressants, including the benzodiazepines, barbiturates with other sedatives and hypnotics (Lishman 1981; Liskow and Goodwin 1987; Baumgartner et al. 1987).
Alcohol toxicity

The toxic effects of ethanol are ascribed to four main mechanisms. They are: (i) an increase in the NADH: NAD ratio and production of H⁺ reducing equivalents (ii) a high rate of production of acetaldehyde, sometimes associated with high blood concentrations (iii) direct toxic effect of alcohol on the hepatocyte (iv) induction of the mixed function microsomal oxidising enzyme system (MEOS) and other microsomal activities leading to a variety of indirect metabolic derangements.

A reduction in the rate of gluconeogenesis, hyperlactacidaemia, hyperuricaemia due to impaired renal excretion of uric acid that result in alcoholics can be attributed to accumulated NADH and excessive reducing equivalents that arise from alcohol dehydrogenase pathway of ethanol metabolism leading to an over-production of lactate and an inhibition of its disposal via pyruvate→glucose metabolism.

An increase in the rate of triglyceride synthesis and production of alcoholic fatty liver, diminished catabolism of fatty acids leading to their enhanced rate of synthesis in promoting hepatic triglyceride deposition are well established. Acetaldehyde, the direct product of ethanol oxidation has been incriminated in disturbances of neurogenic amine metabolism, hepatic glycoprotein synthesis, myocardial protein synthesis and pyridoxal phosphate metabolism. Interestingly, acetaldehyde inhibits albumin synthesis only in the liver of fed animals. In fasted state, this effect is not seen.
The dosage and duration of alcohol consumption probably determines the point in time at which liver converts from an adaptive response to injurious response. Nevertheless, even relatively acute administration of alcohol can result in structural damage to sub-cellular organelles. Seitz et al. (1979) reported that chronic ethanol ingestion produces significant ultrastructural changes associated with altered enzyme activities of subcellular organelles, especially in intestinal smooth muscle endoplasmic reticulum and such changes have been reported by other workers in other tissues in men and in rats (Thayer and Rubin 1986; Kuleilka et al. 1994; Simpson et al. 1994). The impairment of contractility in striated muscle caused by acute and chronic ethanol ingestion was reported (Regan et al. 1966; Rubin et al. 1976; Ohnishi et al. 1984).

Although light microscopic and structural findings of alcoholic liver injury are reported in earlier studies, some of these are non-specific while others provide somewhat insensitive evidence of recent ingestion of alcohol. There is a need to develop reliable plasma markers that are specific for an alcoholic aetiology and which correlate with the degree of histopathological damage (Whitehead et al. 1978). The swelling and distortion of mitochondrial membranes and cristae in alcohol toxicity are accompanied by reduction of mitochondrial enzyme activity and oxidative phosphorylation (Bottenus et al. 1982; Thayer and Rubin 1986; Hirokowa et al. 1998; Sebastian and Setty, 1999). Both alcohol and acetaldehyde have been shown to be capable of inhibiting oxidative phosphorylation. Whatever the mechanism is, the end result is impaired mitochondrial function in general and reduced ability to
regenerate NAD in particular, and inturn a relative failure to metabolise acetaldehyde (Isselbacher 1977; Lieber 1977 a & b).

Chronic ingestion of ethanol results in reduction in the membranes of RER (rough endoplasmic reticulum) as determined by ultramicroscopy, while acute administration is associated with disaggregation of membrane bound polyribosomes and inhibition of albumin synthesis (Isselbacher 1977; Lieber 1977 a & b). Further studies by Oratz et al. (1978) suggested that it is a process of ethanol oxidation to acetaldehyde that is responsible for these effects on the protein synthesizing machinery rather than a critical concentration of either ethanol or acetaldehyde within the hepatocyte. In contrast to defects in hepatic protein synthesis produced by the acute administration of alcohol, there appears to be inhibition of secretion of exportable protein produced by chronic ingestion (Baroana et al. 1977; Sorrell and Tuma 1978; Lieber 1980).

Chronic ethanol consumption may be associated with a greatly exaggerated post-prandial hyperlipidaemia (VLDL), due to a predominant and exaggerated role of the liver in lipoprotein production. It is noteworthy that as the injurious effects of ethanol begin to dominate the adaptive responses, the hyperlipemia disappears. This tends to be associated with a progressive worsening of the fatty liver and development of alcoholic hepatitis (Tavil and Cooksley 1983).

Nirmala and Jeyanthi (1987) have reported that lipid profile such as serum cholesterol, triglyceride and fatty acid may serve as indices of the extent of alcoholic liver cirrhosis. Increased serum triglyceride could be considered as an index of chronic alcoholism (Nirmala and Jeyanthi 1987).
Alcohol induced changes in blood, liver, muscle, brain and heart were reported (Khanna and Madan 1975; Ohnishi et al. 1984; Vasisht et al. 1992; Watanabe et al. 1993; Cronholm 1993; Morell et al. 1998; Dul and Gajkowska 1998).

Hepatocellular failure, a prominent feature in chronic alcohol ingestion is characterised by rise in the concentration of aromatic amino acids, rise in plasma enzymes such as SGOT 2-3 times, in contrast to SGPT which shows minimal degree of elevation. An increase in the ratio of SGOT and SGPT in alcoholic aetiology occurs, whereas a fall in the ratio of the same is noticed in other causes of liver damage. The biochemical basis for this has been reviewed well by Ludwig and Kaplowitz (1980). Elevation in serum gamma-glutamyl transpeptidase has been proved to be a very sensitive indicant of alcohol intake, being significantly raised in normal subjects taking more than two drinks of spirit per day.

Videla and Valenzuela (1982) have reviewed a variety of metabolic changes and pathological alterations in liver and other tissues following alcohol ingestion. An enhancement of hepatic lipo-peroxidation has been reported following acute and chronic ingestion (Koster et al. 1977; Macdonald et al. 1977; Luzio and Stege 1977; Videla et al. 1980; Shaw et al. 1981).

Several studies were carried out on carbohydrate (Ramakrishnan et al. 1976; Kaur et al. 1994; Lands 1997) protein (Preedy and Peters 1989; Ponnappa et al. 1993) and lipid (Pikaar et al. 1987; Vasisht et al. 1992) metabolisms in alcoholics and alcohol administered experimental animals. An increased loss and decreased synthesis of hepatic glutathione in liver was
observed after acute ethanol administration (Speisky et al. 1985). Studies of Yang et al. (1994) suggested that proline and lysine can stimulate ethanol metabolism in prolonged ethanol administered stroke prone hypertensive rats. A decrease in hepatic glucose-6-phosphatase and succinate dehydrogenase and an increase in the activity of glutamate dehydrogenase and phosphopyruvate carboxylase were observed by Ramakrishnan et al. (1976) in rats which were given 30% aqueous alcohol for two months.

Age dependent changes in ethanol metabolism in liver due to diminution in the content of cytochrome P-450 of liver and microsomal functions related to oxidative and few radical mediated reactions namely NADPH oxidase activity, NADPH dependent oxygen uptake and t-butyl hydroperoxide induced chemiluminescence were reported by Fernandez et al. (1988).

**Adaptive responses in alcoholics**

Alcohol is consumed in large quantities over prolonged periods and blood alcohol levels reach maximum concentration in few minutes after ingestion, 20 mM ethanol is considered to be indicative of intoxication (Hoek and Taraschi 1988). Chronic excessive alcohol consumption involves biophysical and biochemical changes in the membranes, and the organism tends to develop a set of adaptive responses to the continued presence of ethanol, presumably, aimed to counteract undesirable effects of this exposure. Such physiological adaptive responses and changes have been demonstrated and confirmed in synaptosomal, mitochondrial, erythrocyte
membranes and also in many other membrane preparations (Chin and Goldstein 1977; Waring et al. 1981; Ellingson et al. 1988).

The adaptive response is detected in membrane preparations isolated from rats that have been fed ethanol over a prolonged period (3-4 weeks) develop blood alcohol levels around 50 mM. Ethanol concentrations of 50-150 mM which causes significant membrane disordering in control preparations have little or no effect in membrane preparations from ethanol fed animals. This adaptive response is referred to as membrane tolerance (Waring et al. 1981; Ponnappa et al. 1982; Hoek and Taraschi 1988).

A Mn\(^{2+}\) dependent peroxidase is induced in Neurospora crassa by ethanol stress (Ramasarma 1994). Another example for such adaptive response is that chronic ethanol ingestion is followed by proliferation of smooth endoplasmic reticulum associated with a proportional increase in cytochrome P-450, cytochrome P-450 reductase and other components of the mixed function oxidase system (MEOS). MEOS activity shows a significant increase as evidenced by the rate of acetaldehyde production and an increase in NADPH consumption (Seitz et al. 1979).

**Alcohol induced changes in membranes**

Ethanol is a drug with profound social implications. By virtue of its lipid solubility it can enter the apolar core of the biomembranes thereby increasing their fluidity, it acts like many anaesthetics, inducing anaesthesia. The pharmacological effects of ethanol are related to its ability to perturb bilayer (Houslay and Stanley 1984). There is now considerable evidence that microsomal, mitochondrial and synaptosomal plasma membranes from brain
and erythrocyte taken from ethanol treated animals are resistant to fluidising effect of ethanol. This is achieved by increasing cholesterol:phospholipid ratio (Chin et al. 1978; Houslay and Stanley 1984; Jain et al. 1988). The intoxicating effect of ethanol on the nervous system can be attributed to its modification of membrane fluidity and alterations of membrane receptors and ion channels. Under pathological conditions, alterations in chemical composition, properties and functions of membranes serve as indices to assess the damage and aetiology of the manifestations (Devlin 1997). Alcohol induced disordering action on membrane under the influence of cholesterol was reported by Johnson et al. (1992). The precise mechanism of action of alcohol on central nervous system is yet to be understood fully (Houslay and Stanley 1984; Peoples et al. 1996). However, several significant changes in the chemical composition, properties, functions of membranes are reported in various models (Chin and Goldstein 1977; Johnson et al. 1979; Goldstein et al. 1982; Crews et al. 1983; Harris et al. 1984; Strong and Wood 1984; Rowe 1985; Rowe 1987; Wood et al. 1987; Stibler and Borg 1987; Wood et al. 1990; Rottenberg et al. 1992; Kaur et al. 1994). Ethanol did indeed fluidise various membranes. Ethanol induced disordering effects in many membranes have been reported in several studies (Chin and Goldstein 1977; Chin et al. 1978; Taraschi et al. 1986). Several morphological, structural and functional changes in membranes of various subcellular organelles have been reported (Waring et al. 1982; Taraschi et al. 1986).
Metabolism of alcohol

The liver is the principal site of ethanol oxidation. There are three pathways of ethanol oxidation in the hepatocyte (Fig.1) localised in different organelles as follows (Tavil and Cooksley 1983; Harris and Crabb 1997):

First Path Way

(1) The cytosolic compartment contains the enzyme alcohol dehydrogenase which catalyses the first step by generating NADH.

\[
\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ \rightarrow \text{CH}_3\text{CHO} + \text{NADH} + \text{H}^+
\]

(2) The second step, catalysed by aldehyde dehydrogenase also generates NADH but occurs largely in mitochondrial matrix space. Liver disposes off NADH generated by these reactions by the only pathway-mitochondrial electron transport chain.

Intake of even moderate amounts of ethanol generates too much NADH. Many enzymes for example, several involved in gluconeogenesis and fatty acid oxidation are sensitive to product inhibition by NADH. Thus during alcohol metabolism these pathways are inhibited, and, fasting hypoglycemia and accumulation of hepatic triacylglycerols (fatty acid) are consequences of alcohol ingestion. Lactate can accumulate as a consequence of inhibition of lactate gluconeogenesis and can result in metabolic acidosis.
Oxidation of ethanol in the hepatocyte and link of the two metabolites (acetaldehyde and H) to disturbances in intermediary metabolism, including abnormalities of amino acid and protein metabolism. NAD denotes nicotinamide adenine dinucleotide; NADH, reduced NAD; NADP, nicotinamide adenine dinucleotide phosphate; NADPH, reduced NADP; MEOS, the microsomal ethanol oxidizing system; and ADH, alcohol dehydrogenase. The broken lines indicate pathways that are depressed by ethanol. The symbol -· denotes interference or binding by the metabolite.

Second Path Way

The microsomes of smooth endoplasmic reticulum contain MEOS (microsomal ethanol oxidising system). This system offers a means for disposing H+ equivalents in presence of molecular O2 by the simultaneous oxidation of NADPH and ethanol to acetaldehyde, water and NADP+. This MEOS is induced after repeated exposure to ethanol and it converts ethanol to acetaldehyde as third route of ethanol catabolism.

\[
\text{CH}_3\text{CH}_2\text{OH} + \text{NADPH} + \text{H}^+ + \text{O}_2 \rightarrow \text{CH}_3\text{CHO} + \text{NADP}^+ + 2\text{H}_2\text{O}
\]

Third Path Way:

The peroxisomes contain the enzyme catalase which is capable of peroxidation of ethanol to acetaldehyde and water in the presence of hydrogen peroxide. The hydrogen peroxide is generated in smooth endoplasmic reticulum by NADPH oxidase utilising hydrogen equivalents and molecular O2 for the formation of NADP+.

NADPH Oxidase – Catalase System

1. \( \text{NADPH}+\text{H}^+ + \text{O}_2 \xrightarrow{\text{NADPH oxidase}} \text{NADP}^+ + \text{H}_2\text{O}_2 \)
2. \( \text{C}_2\text{H}_5\text{OH}+\text{H}_2\text{O}_2 \xrightarrow{\text{catalase}} \text{CH}_3\text{CHO} + 2\text{H}_2\text{O} \)

Acetaldehyde is then oxidised to acetate by a mitochondrial enzyme aldehyde dehydrogenase which is also NAD+ linked. Acetate formed from ethanol is activated to acetyl CoA. The acetyl CoA formed is ultimately oxidised via citric acid cycle for the energy yield. Alcohol has high energy content yielding 7.1 k.cal./g on oxidation. Hence, the term empty calories for
alcohol is misleading (Lehninger 1987). This reaction requires GTP, a product of the succinyl CoA synthetase reaction. The TCA cycle and therefore GTP synthesis are inhibited by high NADH levels during ethanol oxidation.

Much of the acetate made from ethanol, escapes the liver to blood. Virtually every other cell with mitochondria can oxidise it to carbon dioxide by the way of TCA cycle. Acetaldehyde, the intermediate in the formation of acetate from ethanol can also escape from the liver. Acetaldehyde forms covalent bonds with functional groups of biological important compounds. Formation of acetaldehyde adducts with proteins in tissues and blood of animals and humans drinking alcohol has been demonstrated. Such adducts may provide a marker for past drinking activity of an individual.

**Diabetes and Alcoholism**

Diabetes mellitus is a chronic heterogenous group of disorder (Kowsalya et al. 1996) characterised by derangements in carbohydrate, fat and protein metabolism and is associated with many medical complications such as coronary heart disease, retinopathy, nephropathy and neuropathy etc., (Tavil and Cooksley 1983). As alcohol is widely consumed by majority people all over the world, including diabetics, the above said problems associated with diabetes are further complicated in alcoholics (Franz 1983). Keeping the prevalence of diabetics (ICMR Bulletin 1993) and also alcoholism (Bellen 1998) in view, many global organisations and Institution of Diabetic Centre, Minneapolis have issued guidelines for diabetics who consume alcohol (Franz 1983).
An increase in the content of phosphatidylinositol, phosphatidyl-
ethanolamine, phosphatidylserine, phosphatidic acid and appearance of
lysolipid in erythrocyte membrane of diabetics was reported by Freyburger et 
al. (1989).

Christiansen et al. (1994) reported that alcohol accounts for 4 to 6 per
cent of average energy intake in most countries and alcohol induced
hypoglycemia is a well known feared complication in insulin dependent
diabetic subjects but in non-insulin dependent diabetics the amount of
alcohol with a light meal, conditions chosen to mimic an every day situation,
doesn't elicit hypoglycemia.

Alcohol induced hypoglycemia in fasting subjects, and its mechanism,
direct suppression of hepatic gluconeogenesis and increased peripheral
glucose utilisation, alterations in aminoacid levels were reported in diabetics
following the acute administration of ethanol (Frienkel et al. 1965; Kalkhoff
and Kim 1973; Walsh and O'Sullivan 1974; McMonagle and Felig 1975;
Nikkila and Taskinen, 1975; McDonald 1980; Franz 1983). The earlier
studies on diabetics associated with alcoholism were confined to liver and
other tissues (Clark et al. 1965; Phillips and Sarfit 1971; Kalkhoff and Kim
1973; Nikkila and Taskinen 1975; Franz 1983; Christiansen et al. 1994). In
diabetes and other pathological manifestations, significant changes in
chemical composition, properties and functions of membrane, and erythrocyte
membrane in particular, are seen. These changes are prominent in
erythrocytes of diabetics (Gandhi and Chowdhury 1980; Selvam and
Anuradha 1987; Christiansen et al. 1994). Though some earlier work was
done separately in diabetics and alcoholics no data is available on biochemical changes in diabetic erythrocytes associated with alcoholism.