Alcohol is a frequently misused psychoactive drug throughout the world. Alcoholism with its adverse effects ranks among the major current threats to the health and safety of people in the world\textsuperscript{1}. Nearly 100 million people all over the world are reported with diagnosable alcohol related disorders leading to 2 million deaths and 58.3 Disability Adjusted Life Years (DALY) \textsuperscript{2}. Alcohol consumption was linked to more than 60 disease conditions in a series of recent meta-analyses\textsuperscript{3}. Though moderate alcohol consumption has some health benefits, it may lead to chronic excessive consumption resulting in a variety of disorders\textsuperscript{4}. Accumulated literature as well, most health professionals agrees that alcohol affects practically every organ in the human body\textsuperscript{5}. A number of diseases are by definition fully attributable to alcohol\textsuperscript{2}. Further in many diseases a major contributory role of alcohol is confirmed. A sudden increase in alcoholic women disease in recent years raises the broader issues of role of advertising of alcohol and the availability of alcohol drinks in many common places. Advertising of many alcoholic drink notably spirits has been aimed particularly at women\textsuperscript{6}. The study of alcohol use and its consequences at the country level has led to a growing body of knowledge about differential consumption patterns that reflect differences in culture, tradition, religion, social position, income, occupation, gender, region, and a host of other factors\textsuperscript{7}.

In view of the severity of alcohol associated health problems, World Health Organization (WHO) has been developing a standard reference source of information for global epidemiological surveillance of alcohol use since 1966, and research on alcohol\textsuperscript{2} for the past several decades provided much information related to biochemical pathways, alcohol related diseases and mechanisms with current concepts and updated
knowledge. Scientific literature mapping clearly indicated that females (human and animals) were not chosen as experimental subjects for the several toxicological, pharmacological and biochemical studies. As a result many facts related to the impact of gender are not known. Alcoholism has been perceived as a male problem till now. Now the number of female alcoholics is on increase world wide including India and the researchers have become conscious of women related problems as evidenced by the growing number of studies concerning the sex specific ramifications of alcohol use. As female alcoholism was not thoroughly studied so far, now the need is felt to understand the impact of gender related to alcoholism and various physiological as well biochemical events. In view of the changed world scenario the number of female alcoholics has been dramatically increased in various countries including India. An increased number of female alcoholics with disorders who turned up for treatment recently at NIMHANS at Bangalore is evident. Moreover various aspects related to biochemistry and physiology in females with alcohol intake are currently receiving attention. Emerged evidences and reports revealed that females respond differently to alcohol for which several reasons were attributed. Clear assessment of the molecular events as well mechanisms pertaining to toxicity differences with respect to gender facilitates the design of therapeutic strategies. Since literature related to alcohol research is vast and involves diversified areas, the essential information relevant to present topic is furnished below.

**Alcohol and its metabolism**

Ethanol (ethyl alcohol) is the chief constituent in several alcoholic beverages such as beer, wine, whisky etc and is responsible for characteristic effects of alcohol. Though energy researchers, Atwater and Benedict showed that alcohol did produce
7 k.cal/g in the body, alcohol's status as nutrient is more questionable. While small amounts of alcohol acts as a drug producing euphoria for some people, in general it is addictive with the characteristics of tolerance, dependency, withdrawal symptoms and toxicity. Certainly alcohol is a substance available in the diet, but it does not meet the definition of a nutrient. Beer, wine and liquor have different alcohol levels. Most beers have up to 5 percent alcohol, (although some beers exceed 6 percent). Wine 8 to 14 percent alcohol and hard liquor is typically 35 to 45 percent alcohol. Beer and wine are labeled with the percentage of alcohol, but hard liquor is labeled by "proof" which is twice the alcohol percentage (an 80 proof whiskey is 40 percent alcohol).

Consumed alcohol requires no digestion prior to absorption and absorption begins immediately in the mouth and esophagus where small quantities enter the bloodstream. Although alcohol absorption continues in the stomach, the small intestine efficiently absorbs most of the alcohol a person consumes. 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 alcoholic beverage like whisky or beer and about a level of 20 mM ethanol in blood is indicative of intoxication. When equal amounts of alcohol is consumed by man and woman, blood alcohol concentration in woman rapidly raises to the maximum when compared to men in general. 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. About 80% of alcohol absorbed is oxidized in the liver and remaining 10% is excreted in breath, urine, sweat, saliva, milk, tears, and faeces. However in the digestive tract, mainly in the stomach alcohol diffuses into gut cells and travel via portal vein.
directly to the liver where most alcohol metabolism takes place. On the other hand alcohol is subjected to renal clearance. The body works extra hard to get rid of it and quickly metabolises and it removes it from blood. The liver selectively metabolises alcohol and there exists alternative pathways to handle excess consumption. Mitochondrial alcohol dehydrogenase, a zinc containing enzyme converts alcohol to acetaldehyde and aldehyde dehydrogenase quickly effectively converts acetaldehyde to acetate. When large amounts of alcohol prevail, the microsomal ethanol oxidising system (MEOS) operates at a faster speed to process alcohol quickly and converts it to acetaldehyde. Hence this route is an overflow pathway. The peroxisomes contain the enzyme catalase which is capable of peroxidation of ethanol to acetaldehyde and water in presence of hydrogen peroxide. The hydrogen peroxide is generated in smooth endoplasmic reticulum by NADPH oxidase utilizing hydrogen equivalent and molecular O₂ for the formation of NADP⁺.

\[
\text{NADPH} + \text{H}^+ + \text{O}_2 \rightarrow \text{NADP}^+ + \text{H}_2\text{O}
\]

\[
\text{C}_2\text{H}_5\text{OH} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{CHO} + 2\text{H}_2\text{O}
\]

Acetaldehyde is then oxidized 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 oxidized via citric acid cycle for the energy yield. Alcohol has high energy content yielding 7.1 k.cal/g on oxidation. Much of the acetate formed from ethanol escapes from the liver and enters into blood. Virtually, every other cell with mitochondria can oxidize it to CO₂ 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 biologically 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\textsuperscript{24}. Recent studies have suggested that acetaldehyde directly participates in the pathogenesis of alcoholism\textsuperscript{18, 25, 26}. Chronic administration of acetaldehyde during 3 weeks induced metabolic tolerance to ethanol\textsuperscript{27}.

**Characteristic effects of alcohol**

Ethanol is one of the most abused substances and its consumption may lead to addiction. Alcohol short term effects are related to how much a person drinks. One or two drinks bring blood alcohol levels to 0.04\% and usually cause only mild, pleasant changes in mood and release of inhibitions. With more drinks and rising blood alcohol levels, coordination, judgement, reaction time and vision are increasingly impaired\textsuperscript{17}. In many states and Canada, it is illegal for a person whose blood level of alcohol has reached or exceeds 0.08\% to drive a motor vehicle. A recent review of 112 studies concludes that certain skills required to drive a motor vehicle can become significantly impaired at blood alcohol concentration (BAC) as low as 0.05\%\textsuperscript{28}.

Consumed alcohol enters every organ system through the blood stream and affects all the tissues especially brain, heart, liver and gastro-intestinal tract. As alcohol through circulation reaches the brain immediately after consumption as the alcohol concentration rises different parts of the brain are affected and the effects of alcohol on brain\textsuperscript{17} are shown in figure. 1 and Table 1.
HOW ALCOHOL ATTACKS THE BRAIN
A guide to the sequential damage alcohol inflicts on neural tissue

1. First, alcohol affects the forebrain and assaults motor coordination and decision making.

2. Then, alcohol knocks out the midbrain, and you lose control over emotions and increase chances of a blackout.

3. Finally, alcohol batters the brainstem as it affects heart rate, body temperature, appetite and consciousness, a dangerous and potentially fatal condition.

Fig 1. Effect of alcohol on brain
Table 1. Effect of blood alcohol concentrations on brain

<table>
<thead>
<tr>
<th>Blood alcohol concentration</th>
<th>Effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05%</td>
<td>Frontal lobe sedation-reasoning and judgement impaired</td>
</tr>
<tr>
<td>0.10%</td>
<td>Speech and vision center sedation-impaired coordination, vision, driving</td>
</tr>
<tr>
<td>0.15%</td>
<td>Voluntary muscle control impaired-staggering gait, slurred speech, blurred vision</td>
</tr>
<tr>
<td>0.20%</td>
<td>Inability to walk</td>
</tr>
<tr>
<td>0.30%</td>
<td>Stupor, confusion</td>
</tr>
<tr>
<td>0.40-0.60%</td>
<td>Unconsciousness, cardiac or respiratory failure</td>
</tr>
</tbody>
</table>

Alcohol is a simple drug with complex actions and responses that are influenced by hormonal milieu as well as inherent neurobiological differences. Devaud et al.\(^\text{10}\) suggested that a complex series of integrated responses across several brain areas are involved in the development of alcohol dependence and expression of withdrawal symptoms, and that men and women display differential GABAergic and glutamatergic systems that involve the hormonal milieu, as well as sexual dimorphism of the brain.

Alcohol readily crosses protective fatty membrane of nerve cells and disrupt brain complex system for communicating between cells. Besides release of different neurotransmitters that cause loss of balance, sleepiness, loss of coordination, imbalance of functions, impairing judgment and mental ability\(^\text{17}\). Further some neurotransmitter perpetuate the desire to keep drinking and also lead to addiction and symptoms of alcohol withdrawal. In short term they probably contribute to a hangover\(^\text{29}\).

Ethanol is a central nervous system depressant that decreases the activity of neurons, although some behavioral stimulation is observed at low blood levels. This drug has cross tolerance and shares a similar pattern of behavioral problems with other
brain depressants, including the benzodiazepines, barbiturates with other sedatives and hypnotics\textsuperscript{30-33}.

Long term alcohol use affects almost every organ system of the body, potentially resulting serious illness including liver disease, impaired heart function, inflammation of pancreas and neurological disorders\textsuperscript{34}. Investigating the mechanisms underlying these adverse effects of alcohol consumption in humans frequently is impractical, because alcohol related disease generally develops only after many years of heavy drinking\textsuperscript{35}. Furthermore many studies would be unethical to conduct in humans. Therefore researchers have been forced to use various animal models to gain insight into the processes responsible for alcohol's effects on the body and to determine new ways of preventing or treating alcohol related diseases\textsuperscript{35}. Among several disorders and diseases alcoholic liver disease (ALD), cardiovascular disease (CVD), coronary heart disease (CHD), brain damage and some neurological disorders are considered to be closely associated with chronic alcoholism\textsuperscript{35}. These pathologies generally affect both genders. Alcohol induced changes in blood, liver, muscle, brain, and heart were reported\textsuperscript{6-43}. Hepatocellular failure, a prominent feature in chronic alcohol ingestion is characterized by rise in the concentration of aromatic amino acids, rise in plasma enzymes such as serum glutamate oxaloacetate transaminases (SGOT) 2-3 times, in contrast to serum glutamate pyruvate transaminases (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 cause of liver damage. The biochemical basis for this has been reviewed well by Ludwig and Kaplowitz\textsuperscript{44}. Elevation in serum gamma-glutamyl transpeptidase has been proved to be a very sensitive indicator of alcohol intake, being significantly raised in normal subjects taking more than two drinks per day. Mothers who drink heavily during pregnancy have an
increased risk of bearing children with fetal alcohol syndrome (FAS) or other alcohol related birth defects*. It has been established for many years that hepatic mitochondria are appreciably altered both in structure and function as a consequence of chronic ethanol consumption. The changes are accompanied by alterations in both the polypeptide and lipid composition of mitochondrion45.

To examine the effects of chronic alcohol administration on mitochondria, the total cholesterol and phospholipid contents in ethanol fed hepatic mitochondria of female rats were compared with other groups. The phospholipid alternation leads ultimately to a structural disaggregation of mitochondrial membranes45. Lopez46 has studied the formation of co-polymers of proteins and lipids after the induction of peroxide reactions of unsaturated fatty acids. Early studies also revealed that phospholipids play a key role in alcohol treated mitochondria related to fluidity influencing the physiological functions of the membrane46.

Gender based differences in metabolic regulation and certain biochemical events in response to alcohol in male and females have been reported. Such gender differences in the incidence of hyperlipidemia, CHD and differential body glucose utilization are well documented46-51. Studies of Zhang et al.52 also confirmed such impact of gender by their experimentation using the effect of garlic oil (GO) on plasma cholesterol and glucose levels of normal subjects. They demonstrated that women showed favorable effects in terms of CHD risk factors (i.e. increases in HDL-C and reduction in TC/HDL-C) where as men had small adverse effects.49 There was a significant difference in the garlic oil effect for glucose with reduction seen for men and increase for women51. The authors did not expect such analyses which were not
planned in advance and this led to recognize the importance of the gender for designing future studies by the authors\textsuperscript{52}.

Lisa et al.\textsuperscript{53} showed such gender differences in alcohol using adolescents with a higher risk for developing behavior impairments and alcohol used disorders along with BOLD (Blood Oxygen Level Dependent) response related to brain affecting the performance. Reactive oxygen species play an important role in ethanol induced intoxication, in general due to lack of pro-oxidant activity and potential antioxidant ability\textsuperscript{54}. Alcohol consumption is known to stimulate early steps in reverse cholesterol transport reflecting changes in plasma lipoprotein cholesterol levels\textsuperscript{55}. Despite the fact that most of the earlier work on alcohol consumption has been conducted on men or mixed gender groups, it is particularly important to examine the data for men and women separately. Women typically consume alcohol less frequently and smaller amounts than men\textsuperscript{6}. Yonker et al.\textsuperscript{56} reported that moderate alcohol may be beneficial in cognitive function in women, but not necessarily in men. Since the late 1980's drinking amongst women, and particularly among the young, has increased at a greater place than in men\textsuperscript{57}. If drinking levels in women approach those of men, then we can expect the rates of drink problems amongst women will overtake those in men because of their physiological sensitivity to the effects of alcohol\textsuperscript{58}. The greater social acceptance in men compared to women may explain why fewer women than men drink excessively, even when physiologically equivalent alcohol levels are compared\textsuperscript{15}. The mechanisms that underlie and influence the onset, progression and severity of alcohol related disease are not well understood.
Effects of alcohol on Liver

Liver is a hormone sensitive organ and plays a vital role in maintaining the internal environment (the homeostatic balance) in higher organisms and is affected by alcohol abuse. Alcohol is chiefly metabolized in liver. Alcohol affects the liver acutely and after long term exposure depending on the extent of alcohol abuse, alcoholic liver disease occurs in 3 stages from fatty liver to alcoholic hepatitis (steatohepasis) and finally to cirrhosis. It is well known that liver is the primary site of alcohol metabolism in the body. Clinical biochemical abnormalities associated with this disorder includes elevated levels of aspartate and alanine transaminases, alkaline phosphatase and higher \( \gamma \)-glutamyl transpeptidase. The free radicals generated during metabolism of ethanol cause the direct cell damage that occurs during alcohol liver disease. In general antioxidants scavenge those free radicals preventing the tissue from damage. Chronic alcohol consumption diminishes the levels of the antioxidants and makes liver cells more susceptible to free radical induced injury. Further the hypoxia which arises due to increased oxygen utilization by liver cells for metabolizing alcohol and making oxygen non available for cellular functions contributes for the damage. Inflammatory agents, eicosonoids and cytokines contribute to hepatic damage. Nirmala and Jeyanthi reported that lipid profile such as serum cholesterol, triglyceride and fatty acid may serve as indices of the extent of alcoholic liver cirrhosis. 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 incriminate 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., reported that chronic ethanol ingestion produces significant ultra structural 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. The impairment of contractility in striated muscle caused by acute and chronic ethanol ingestion was reported. 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 which correlates with the degree of histopathological damage.

The swelling and distortion of mitochondrial membranes and cristae in alcohol toxicity are accompanied by reduction of mitochondrial enzyme activity and oxidative phosphorylation. 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 in turn a relative failure to metabolize acetaldehyde.

Chronic ingestion of ethanol results in reduction in the membranes of rough endoplasmic reticulum (RER) as determined by ultra microscopy, while acute
administration is associated with disaggregation of membrane bound polyribosomes and inhibition of albumin synthesis. Further studies by Oratz et al. 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.

Chronic ethanol consumption may be associated with a greatly exaggerated post-prandial hyperlipidemia (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 hyperlipidemia disappears. This tends to be associated with a progressive worsening of the fatty liver and development of alcoholic hepatitis. Videla and Valenzuela 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.

Several studies were carried out on carbohydrate, protein and lipid 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. Studies of Yang et al. 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. 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. Differences in liver’s response to both acute and chronic consumption of alcohol in men versus women are documented. There are sex differences in location and quantity of hepatic alcohol dehydrogenase (ADH) and in the first pass metabolism of ethanol. Alcohol induced suppression of gluconeogenesis is greater in ethanol fed female rat hepatocytes than in males. Chronic alcohol consumption resulted in a decrease in glucose appearance and more importantly specific decline in hepatic gluconeogenesis capacity in the absence and presence of ethanol. An early decline in the whole body glucose appearance in females fed with an ethanol diet in comparison to controls was observed. While the decline was also observed in males fed with the alcohol diet, it occurred much later compared to ethanol-fed females. Decreased hepatic gluconeogenesis production of glucose from lactate in ethanol fed females was reported. However the mechanism for the specific decline is not known. Alcoholic women appear to be more susceptible to ethanol induced hypoglycemia compared to alcoholic men. However many mechanisms and events related to glucose homeostasis pertaining to gender are yet to be established.

**Alcohol induced biochemical adaptive changes in membranes**

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. 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 conformed in synaptosomal, mitochondrial, erythrocyte membranes and also in many other membrane preparations\textsuperscript{102-104}. The adaptive response is detected in membrane preparations isolated from rats that have been fed ethanol over a prolonged period (3-4 weeks) and had blood alcohol levels around 50 mM. Ethanol concentrations of 50-150 mM, which cause 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\textsuperscript{20,34,35,105}.

A Mn\textsuperscript{2+} dependent peroxidase is induced in \textit{Neurospora crassa} by ethanol stress\textsuperscript{106}. Another example for such adaptive response is that chronic 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\textsuperscript{21}.

By virtue of its lipid solubility ethanol can enter the polar core of the biomembranes thereby increasing their fluidity. It acts like many anesthetics including anesthesia. The pharmacological effects of ethanol are related to its ability to perturb bilayer\textsuperscript{107}. There is now considerable evidence that microsomal, mitochondrial and synaptosomal plasma membranes from brain and erythrocyte taken from ethanol treated animals are resistant to fluidizing effect of ethanol. This is achieved by increasing
cholesterol: phospholipid ratio. 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. Alcohol induced disordering action on membrane under the influence of cholesterol was reported by Johnson et al. The precise mechanism of action of alcohol on central nervous system is yet to be understood fully. However, several significant changes in the chemical composition, properties, functions of membranes are reported in various models. Ethanol did indeed fluidize various membranes. Ethanol induced disordering effects in many membranes have been reported in several studies. Several morphological, structural and functional changes in membranes of various sub-cellular organelles have been reported.

Alcohol vs Neuroendocrine system and Brain

Alcoholism affects endocrine functioning and disrupts normal growth and endocrine development through its effects on hypothalamus, pituitary gland and various targets organs such as ovaries and testis. Moreover bone density and growth are affected. Devaud et al. have shown significant differences between the sexes in behavioral responses and neuroadaption to chronic alcohol consumption and withdrawal. Accumulated evidences suggested that treatment of alcoholism, including withdrawal, should be tailored to the patient’s gender and hormonal status. Sex differences in brain and endocrine mediated stress processes in alcoholics are evident while flight or fight response is seen in males, women often evince tend or befriend behaviors, which is rather than responding aggressively to a challenge, women are more likely to
nurture in others in stressful situation. Further, women exhibit a greater release of stress hormone cortisol in response to stress compared with men suggesting there is a greater physiological response in females compared with males. Another major physiological parameter affected by alcohol is the hypothalamus-pituitary-gonadal (HPG) axis, which regulates the synthesis of gonadal steroids in both males and females. The hypothalamus produces gonadotropin-releasing hormone (GnRH), which travels to the pituitary, where it stimulates the release of both luteinizing hormone (LH) and follicle stimulating hormone (FSH) into the blood stream. LH and FSH control testosterone synthesis and secretion as well as sperm development in the testes of male animals and follicular rupture and ovulation in females. In males, GnRH is secreted in pulsatile, noncircadian manner. In females, the hormone is secreted in a cyclical manner throughout the menstrual or estrus cycle in response to changing levels of estradiol and progesterone secreted from the ovaries. Alcohol detrimental effects on developing brain as well adult brain is the most serious concern. It is now well established that even uncomplicated alcoholics who have no specific neurological or hepatic problem show signs of brain damage and cognitive system. Recent studies using neuroimaging technology, magnetic resonance spectroscopy, positron emission tomography contributed significantly for understanding such changes. Further DNA micro-arrays and proteomics might also provide clues to this important problem.

Alcohol vs cardiovascular effects:

Hyperlipidemia associated with alcohol consumption is relevant to the problem of atherosclerosis and heart disease in the drinking population. Elevated serum triacylglycerol, LDL-C and decreased HDL-C were reported to be risk factors for cardiovascular disease. Excessive alcohol consumption has long been associated with
cardiovascular disorders, including cardiomyopathy, hypertension, coronary artery disease and stroke. However, recent evidences suggested that moderate intake of alcohol provides of cardio-protection, particularly against coronary heart disease and ischemia-reperfusion injury. 140. 13 million Americans alone are affected by coronary heart disease.141.

Atherosclerosis is a diffuse disease, and its presentation varies depending on vascular bed in which it occurs. In the coronaries an acute clinical event occurs by the induction of plaque rupture in lipid rich regions. Plaque rupture leads to thrombus formation, partially or complete occlusion of the arteries, unstable angina, myocardial infraction, and/or sudden death, similarly in carotid arteries peripheral vasculature. These events occur often leading to thrombotic stroke.142. The compounds of inflammatory and thrombotic pathways are influenced by alcohol. Hypercholesterolemia, inflammation, and thrombosis caused by a hypercoagulable state are part of the complex etiology of the atherogenic process.143.

HDL-C has been shown to protect against atherosclerosis by influencing a number of steps in the initiation and progression of the disease. HDL-C inhibits LDL-C oxidation, thus preventing uptake by macrophages, foam cell formation and apoptosis.144. Though the precise mechanism of ethanol induced pancreatic damage is unknown, evidences suggested that non-oxidative metabolites of ethanol rather than ethanol itself are responsible for the marked elevation of Ca²⁺ that mediate toxicity in the pancreatic acinar cell.145 A non immunological mechanism, possibly mediated by a soluble variant of human asialoglycoprotein receptor and mechanical share stress are involved in hemolysis in chronic alcoholics.146. Seo et al.,147 suggested that chronic alcoholism contributed to the increased risk of vascular diseases in Koren alcoholics.

27
Changes in eNOS regulatory pathways after chronic alcohol intake lead to alcohol mediated disruption of the liver microcirculation causing alcohol induced hypersensitivity to (lipopolysaccharide) LPS and endothelin-1 (ET-1) \textsuperscript{145-149}. Tirepelli \textit{et al.},\textsuperscript{150} have postulated that the gender specific vascular effects elicited by chronic consumption in rats are not related to ovarian hormones but seemed to involve the upregulation of iNOS.

Levels of the non-oxidative products of ethanol metabolism viz., fatty acid ethyl esters are elevated in various tissues causing organ damage. Accumulation of saturated fatty acid ethyl esters of long chain fatty acids in maternal and fetal organ in alcoholic pregnant women is reported\textsuperscript{151}. Ethanol consumption is also associated with increased triglycerides and HDL-C in liver and serum\textsuperscript{152}. Moreover ethanol causes increased total as well as plasma membrane cholesterol thereby altering membrane fluidity with a consequent decrease in Ca\textsuperscript{2+}, Na\textsuperscript{+}, K\textsuperscript{+}-ATPase activities\textsuperscript{153}. Depressed oxidative capacity of mitochondria and development of hyperlipidemia with liver injury are well known\textsuperscript{154}. A derailment in lipoprotein metabolism with accumulation of LDL and VLDL, the main risk factors of atherosclerosis, are reported\textsuperscript{154}. Chronic ethanol intake leads to oxidative modification of hepatic mitochondrial protein thiols as well cytosolic protein carbonyls. Accumulation of damaged proteins play a pathogenic role in alcoholic liver disease and other related diseases\textsuperscript{155}. The protein adducts are seen in the centrio-lobular region of the liver in alcohol abusers\textsuperscript{156}. Deposition of acetaldehyde adducts and many hybrid adducts such as aldehyde products of lipid peroxidation aggravates ethanol toxicity in liver, gut, muscle, brain and heart\textsuperscript{157,158}. Alcohol also influences the regulation of production as well activities of a variety of enzymes such as xanthine oxidase, CYP2E1, Mn\textsuperscript{+}, superoxide dismutase etc. Lipid peroxidation, superoxide production and generation of hydroxyl ethyl radical occur. Metabolism of
ethanol is linked with stimulation of kupffer cells, activation of NF-kB, production of TNF-α, free radical generation and oxidative stress. Nebbia et al. reported significant sex related differences in the basal CYP2E1 expression and catalytic activity as well as in the susceptibility to Phenobarbital induction between lines of rats selectivity bred for their respective preference (P) or aversion (NP) for drinking ethanol. Studies of Crippens et al. revealed that circulating ovarian hormones do not influence alcohol distribution to brain, but do influence distribution to more peripheral tissues such as the tail. Moreover, apparent differences in tail blood alcohol levels may not reflect differences in brain levels. Ethnic and gender differences in ethanol metabolism with lower metabolism of alcohol in Asian women were reported. This was attributed to lesser ADH and ADH isozymes activities. Jung et al. based on their studies on rats suggested that the development of separate clinical strategies for the treatment of anxiety disorders related with ethanol abuse in men and women may be important.

**Women and alcohol**

Reports clearly revealed that alcohol consumption by women has increased worldwide to a large extent. Besides studies demonstrated that men and women respond differently to alcohol. Blood alcohol rises faster in women. So they become more intoxicated than men with an equivalent dose of alcohol. Accordingly moderate drinking is usually defined as “two standard drinks for men and one for women”.

Roman reported that a greater physiological deterioration among women as compared with man who have similar drinking histories. Fetal alcohol syndrome (FAS) is a major problem associated with women drinking behavior. The dosage and duration of alcohol ingestion probably determines the point in time at which the liver converts...
from an adaptive response to an injurious response. Nevertheless, even relatively acute administration of alcohol can result in structural damage to subcellular organelles. Experimentally, in animals and man, ultra structural changes have been noted in mitochondria and in smooth and rough endoplasmic reticulum. In the case of the SER these have been identified as hypertrophy of the machinery concerned with ethanol metabolism. Both alcohol and acetaldehyde have been shown to be capable of inhibiting oxidative phosphorylation. Whatever the mechanism is, the result is impaired mitochondrial function in general, and reduced ability to regenerate NAD in particular, and in turn, a reactive failure to metabolize acetaldehyde. Women metabolize alcohol more slowly than men. Several factors are responsible for alcohol’s greater effect on women.

1. **Body size and composition** Alcohol has a smaller volume of distribution in women because adipose tissue into which it diffuses slowly because of the poor blood supply forms greater proportion of body mass in women (33%) than in men (21%). Man on average tends to have more body water for distribution of alcohol whereas women generally have smaller volumes of distribution for water soluble drugs such as ethanol which may account for higher blood alcohol concentrations (BAC) of women at comparable quantities. There is paucity of information regarding the precise gender based requirements of water.

2. **Differential enzyme activities and metabolism** Women metabolize less ethanol than men because of lower levels or less activity of gastric ADH. The size of liver in females was reported to be smaller in comparison to males. Differences in metabolism and elimination are important considerations Baraona et al. (2001). Alcohol pharmacokinetics during menstrual cycle leading
to slightly faster elimination of alcohol during luteal phases and women’s drinking which appear to be hazardous and remain unverified. In general, women tend to drink more slowly than men. There is very little information on to what extent women and men consume food with alcohol. Concurrent use of other substances may alter the effects of alcohol. There is huge gap in our knowledge on this matter. All these things are to be taken into considerate when comparing the effects of alcohol on women and men. Hence there is also a need for in depth research on behavioral factors affecting how alcohol is consumed. Further gender differences in alcohol absorption, metabolism and elimination are to be investigated. Too little is known about how these processes are linked to levels of sex hormones.

3. **Hormonal milieu** In addition to biological (anatomical and physiological) differences, biochemical aspects are influenced by hormones. Studies related to alcoholism reveal that androgens and estrogens influence the effects of alcohol. Female rats release more corticosterone than males in response to alcohol and suggested that those corticosteroids may play a role in the rate of alcohol metabolism. Almela et al. reported the existence of gender differences in alcohol preference and consumption mediated through dihydrotestosterone (DHT1) in rats. Further sex differentiation of brain and sex steroid milieu might account for the typical male and female patterns of alcohol preference and ingestion. Other studies revealed that gene expression changes that are controlled by sex hormones are responsible for greater susceptibility of female gender leading to ethanol induced liver injury. Green et al. have concluded that health may be benefited on moderate drinking more in women than men.
Recent studies clearly revealed the existence of the role of nitric oxide in gender differences\cite{149,150,158,197}.

Horton et al revealed that nutritional state can significantly impart differential postprandial metabolism in men and women with respect to triglyceride uptake which was attributed to sex steroid environments in men vs women\cite{172}. Reversal of sex differences in lipid metabolism in gonodacterized rats were observed by Coleman et al.\cite{173} The effects of gonodacterized much more pronounced in the male than in female animal. Such differences certainly lead to differential response to alcohol by females\cite{174}.

Estrogens are neuroprotective with respect to neurological degeneration growth and susceptibility to toxins. The cyclic fluctuations of estrogens and progesterone enhance response to stress, which confers susceptibility to depression and anxiety\cite{8}. Earlier studies showed that alcoholic liver disease readily develops in women than in men and its progression may also be quicker\cite{170,171,183,188,193,217}. Certain ovarian steroids such as allopregalone, pregalone and progesterone have effect on the brain and brain functions. Further they interfere in the interaction between GABA$_A$ receptor and alcohol. In general allopregalone is a positive modulator of GABA$_A$ receptor with sedative, anxiolytic and anticonvulsant effects in both humans and animals\cite{172}.

4. **Differences in physiology of organs** It is well known that there exists certain differences in structure of brain, the ratio of white matter to gray matter and volume of brain and composition related to brain differences in sex organs, differences in vasculature of different organs such as intestine may also
contribute to the gender differences in alcohol sensitivity and differences in blood alcohol levels (BAL)\textsuperscript{138}. Different cognitive processes are altered by chronic ethanol exposure in male and female rats and the neurobiological mechanisms responsible for these differences remain to be determined.

A few isolated studies, conducted indicated gender differences on differential behaviour of alcohol\textsuperscript{10,128}. This needs to the further assessed from a huge number of studies with use of females as subject. Similarly female alcoholism is not as thoroughly investigated as alcoholism among male subjects\textsuperscript{1}. Owing to emerging evidences on the impact of female gender on alcohol, few researchers have undertaken studies using women and female animal models for research and the design of the experiments, selection of models and doses of alcohol administered were at scientists’ discretion and reporting ambiguous results. Further several researchers have repeatedly emphasized a need of studies on females pertaining to alcoholism\textsuperscript{6,7,11,196-199}. A number of differences exist between the sexes in rates of illness and course of illness with Alzheimer’s disease, schizophrenia, alcoholism and mood and anxiety disorders\textsuperscript{175,176}. An increasing number of women are financially independent and tend to adopt male drinking habits. Hence the incidence of alcoholism is increasing among women\textsuperscript{12,14,15}. Researchers suggest that alcohol affects brain structure differentially in men and women. As there exists differences in the functioning of brain’s two hemispheres, normally differential asymmetries leading to perceptual abilities are evident\textsuperscript{136,176}.

Hommer et al.\textsuperscript{176} showed that alcoholic women have smaller gray and white matter volumes and greater Cerebrospinal fluid volumes than healthy
non-alcoholic women and that the brain volume differences between alcoholic and non-alcoholic women particularly in gray matter are considerably larger than those found between alcoholic and non-alcoholic men. Further it was concluded that women are more vulnerable to many of the medical consequences of alcohol use than men.176

Studies also revealed that women develop hepatic and cardiac complication of alcoholism earlier in the course of disease than men do. According to animal studies female rats also develop severe alcohol induced liver injury than male rats179,183,217. Gubala and Zuba177 showed gender differences in the pharmakinetics of ethanol in saliva and blood after oral ingestion. It is well established that gender directly influences the cardiovascular system. Zheng et al.,178 observed changes in the small intestine vasculature which represent a potential mechanism for the circulatory gender difference between male and females and androgens play an inhibitory role on small intestinal endothelial function. Thurman et al.,179 put forth forwarded mechanisms of alcohol induced hepatotoxicity that involves activation of kupffer cell by endotoxin in rats leading to alcoholic liver injury in which females are more sensitive to these changes. Coelantoni et al. (2003) observed difference in cytokine responses that contribute to the enhanced sensitivity of female liver to ethanol induced injury.180 Enomoto et al.,181 demonstrated that activated kupffer cells are involved in alcohol induced fat accumulation in rat liver by releasing prostaglandin E2. Galuicci et al.,182 observed that differential expression of liver interleukin-6 receptor α in female rats is responsible for alcohol liver disease (ALD). Nanji et al.,183 showed that increased endotoxia and lipid peroxidation in female rats stimulating NFkB activation and
chemokine production (TNF-α, COX2 upregulation) are responsible for enhancement of liver injury in females. Mann et al.,\textsuperscript{184} demonstrated higher vulnerability to alcoholic damage among women and also gender selective effects of chronic ethanol in women leading to adaptation at molecular level.

Studies of Cha et al.,\textsuperscript{185} indicated that sex-dependent and cycle-dependent differences in ethanol sensitivity are manifested at both the behavioral and neurophysiological levels and may have important implications for our understanding of the neurobehavioral effects of ethanol. Susan Nolon-Hoeksema\textsuperscript{9} reported that women appear to suffer more negative consequences in a number of domains than men from drinking a given amount of alcohol. Studies of Jung et al.,\textsuperscript{162} revealed that neuroprotective effects of estrogen treatment against alcoholic damage in men and women. Female susceptibility to the toxic effects of alcohol has been observed in hepatic, cerebral, cardiac and muscular alternations. Similarly females were found to be more sensitive to the toxic effects of alcohol on peripheral nerve fibers and alcoholic neuropathy unrelated to malnutrition.\textsuperscript{162} Alcoholic damage related to all the above mentioned disorders were described in several reviews\textsuperscript{186-195}. Spitzer\textsuperscript{196} found gender differences in NO production by alveolar macrophages in ethanol intoxicated rats. On the contrary Ronis et al.,\textsuperscript{197} ruled out the greater susceptibility to ethanol induced hepatotoxicity in females than in male rats. Hallman et al.,\textsuperscript{15} reported that female alcoholics with and without multiple substance misuse show differences in various biochemical parameters when compared to males. Further such co-morbidity seems to be more frequent among female than among male alcoholics\textsuperscript{147,201}. Crippens\textsuperscript{160} reported the gender differences in blood ethanol level but not in brain. Female rats differ
significantly from males in their mesolimbic dopamine activity as related to ethanol drinking. Myers et al.\textsuperscript{202} opined a major draw back in most experimental studies of ethanol preferring strains of rats is the minimal usage of female gender. Sumida et al.,\textsuperscript{203} reported that chronic alcohol consumption results in gender dependent differences in whole body glucose production and regulation.

**Alcohol vs. nitric oxide**

Nitric oxide is a signaling molecule that had been shown to be involved in diverse cellular functions and is known to mediate various physiological as well pathological processes\textsuperscript{204}. Current literature reveals that the metabolic status of NO and functional status between oxidation and anti-oxidation systems in animal/human body are in close relationship with health. If the metabolism of NO is abnormal and the dynamic balance between oxidation and anti-oxidation is disturbed there will be an increase in free radical concentration. Consequently free radical chain reactions will aggravate the damage in the animal/human body\textsuperscript{205}. Several studies clearly demonstrated the role of NO in alcohol induced events\textsuperscript{148,150}. There is paucity of information concerning the role of NO induced differential response in relation to gender in chronic alcoholism.

The development of alcohol induced liver toxicity is influenced by many mechanisms including changes in metabolism, inflammation and fibrogenesis\textsuperscript{206}. In addition to hypoxia, oxidative and nitrosative stress have been suggested as key factors capable of both initiating and sustaining the mechanisms of pathogenesis leading to alcohol liver disease (ALD)\textsuperscript{207,208}. Further mitochondrial damage plays a significant
role in this process. Cytochrome C oxidase contains an oxygen binding site i.e., the principal target for the interaction of NO in regulating several aspects of mitochondrial function including formation of ROS\textsuperscript{209,210}. NO production, although generally considered beneficial, can become deleterious on reaction with superoxide, resulting in the formation of peroxynitrite\textsuperscript{211}. This occurs in response to chronic ethanol exposure formation of peroxynitrial radical leading to many adverse consequences\textsuperscript{212}.

Now it is well known that nitric oxide regulates virtually every critical cellular function and also mediates cellular damage in a wide range of conditions. NO itself is potentially toxic. Moreover its oxidation products are more toxic. NO often interacts with superoxide thereby forming peroxynitrite. Further studies also revealed the formation of many reactive nitrogen species. Although peroxynitrite is a strong oxidant it reacts at a relatively slow rate with most biological molecules\textsuperscript{213}. Peroxynitrite is able to cross cell membranes through anion channels and reacts slowly and selectively through the cell making the biological and pathological implications. Peroxynitrite favors collective reactions with key moieties in proteins such as thiols, iron-sulphur centers and zinc fingers. Further it interacts with lipids and nucleic acids\textsuperscript{214}. As a result many nitrated products are formed leading to cytotoxicity in broad actions such as inflammation, cardiovascular disease, neurodegeneration, diabetes and other pathologies\textsuperscript{215}. According to a working hypothesis proposed by Limuro et al.,\textsuperscript{216} higher plasma endotoxin levels lead to more extensive kupffer cell activation in females than in males. As a consequence, increase in free radical-producing inflammatory cells in the liver occur. This could explain why females develop alcohol-induced liver injury more rapidly and to a greater extent than males\textsuperscript{216}.
The Kupffer cells of liver and other inflammatory macrophages in liver can aggravate hepatotoxicity of ethanol. Chronic alcohol intoxication of rats causes an increase in macrophages and neutrophils. Moreover ethanol intoxication stimulates some activities of macrophages and neutrophils such as the release of superoxide anion and cytokines, the mediators involved in the pathogenesis of alcohol induced liver disease. Various cell types in liver including Kupffer cells, hepatocytes, and monosides have the ability to synthesize nitric oxide during endotoxaemia and inflammation.

Mitochondrial dysfunction is known to be a contributing factor to a number of diseases including chronic alcohol induced liver injury. Chronic alcohol intake leads to mitochondrial damage, decrease in mitochondria membrane potential and injury and elevated intracellular Ca$^{2+}$ production. Reactive oxygen and nitrogen species disrupt mitochondrial function through post-translation modification of mitochondrial proteome. According to Limuro et al., though many hypotheses have been presented to explain gender differences in response to alcohol, including alternations in absorption, disposition and metabolism. However evaluation of these hypotheses has become difficult because of lack of a clinically relevant model. Nitric oxide, generated by NOS, is implicated in a wide variety of normal and pathophysiological processes ranging from vasodilatation, immunodefence and neurotransmission to atherogenesis and carcinogenesis. Now it is well known that NO is produced in numerous cell types and many isoforms of NO have been recognized among which three types (nNOS, e NOS, and iNOS) are well studied. NO production is increased in response to chronic alcohol via induction of inducible NOS. This has important ramifications for toxicity because NO and its metabolite peroxynitrite (ONOO-) have been implicated as key mediators of mitochondrial dysfunction. NO diffuses into
mitochondria and reacts with $O_2^-$ to produce ONOO$^-$, a reactive metabolite responsible for inactivation of mitochondrial proteins via posttranslational modifications$^{224}$. Nitric oxide produced from different cell types is capable of diffusion to great distances at physiological oxygen tensions in tissue. As such produced NO would be capable of distant endocrine vasodilatation resulting in loss of local metabolic and flow regulated vasi-control$^{24}$. Several studies revealed that NO interacts with various important biomolecules resulting in the formation of nitrated lipids$^{230}$, nitrated proteins$^{231}$, nitrosamines$^{232}$, and iron nitrosyls$^{233}$, etc. Further NO appears to be involved in DNA damage regulation of metabolism and also in several membrane-dependent processes$^{234,235}$. Though the involvement of NO in alcohol-induced events is well documented, its precise role, mechanism, and effects are yet to be understood fully. NO is known to influence the composition, physico-chemical properties as well as functions of blood, liver, heart, nerve system, and brain and also other tissues to a large extent. Several studies indicated a novel role of NO in the pathogenesis of alcohol hepatotoxicity$^{236}$. Loss of control of NO signaling per se results in excessive inhibition of respiratory chain leading to bioenergetic dysfunction (i.e., decreased ATP synthesis) and increased ROS production$^{236,237}$. Now current literature clearly reveals the dual role of NO, a beneficial and detrimental depending on its concentration and physiological conditions. NO is a gas i.e., continuously synthesized in endothelial cells and several other cell types which executes multiple functions that maintain vascular homeostasis$^{238-241}$. Though erythrocytes and plasma are set to be the major reservoirs, its prevalence in brain and other tissues such as liver, pancreas etc., and its novel interactions with mitochondrial proteins and other components reveal a doubt on the former$^{207,212,214}$. 
The iNOS produces more NO compared to eNOS or nNOS\textsuperscript{238}. Increased NO biosynthesis via I NOS (chronic stimulation) may contribute to platelet and endothelial dysfunctions. These changes contribute to the motor disturbances, which are associated with alcohol abuse\textsuperscript{240}. Much of the toxicity of NO may be mediated by forming more potent oxidants like peroxynitrite. Peroxynitrite formation also participates in ethanol induced oxidative stress. Since NO can diffuse freely into the mitochondria, NO reacts rapidly with intramitochondrial superoxide\textsuperscript{241}.
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