DISCUSSION

1) Impact of gender on plasma markers in normal and alcohol administered rats

The current interest in studying the impact of gender on alcoholism transcends from the recent reports on differential biochemical, behavioral and toxicological responses and also sensitivity to alcohol related diseases such as alcohol liver disease (ALD), cardiovascular disease (CVD) and brain damage of females to alcohol induced changes. The data obtained from the present study primarily confirms the existence of gender impact on certain routine plasma metabolites, enzymes and markers in normal rats. The concentrations of glucose, lipid peroxides, nitrite, nitrate and lipids (cholesterol and triglycerides) as well the activities of plasma enzyme markers GGT, SGOT, SGPT and alkaline phosphatase were higher in normal male rats when compared to normal females suggesting the existence of gender impact on these parameters (Table 2). Further, the data demonstrated that alcohol consumption caused an elevation of the levels of the mentioned metabolites and also the activities of enzymes in all the experimental groups, viz., female rats with intake of 4g ethanol/kg b.w/day and male rats with intake of 10g ethanol/kg b.w/day, but not in male rats receiving 4g of ethanol/kg b.w/day. All these changes suggested the pronounced damage caused to liver by chronic alcohol consumption in rat groups receiving 4g ethanol in females/kg b.w/day and 10g ethanol in males/kg b.w/day. However 4g ethanol/kg b.w/day did not induce any significant damage in male rats.

Histopathological evidences of the present study confirmed the observation drawn from above biochemical analyses. It is evident from these observations that 4g ethanol/kg b.w/day supplementation in females and 10g ethanol/kg b.w/day supplementation in males induced toxic changes leading to pathogenesis but not in 4g
ethanol/kg b.w/day receiving male rats. Hence the interest of the present study is to understand the mechanisms associated with alcohol induced adverse effects in female rats with respect to gender impact. In further experiments of the present study female rats with 4g ethanol intake /kg b.w/day and males with and 10g ethanol intake/kg. b.w/day were used to compare them with respective controls. Though no systematic study was conducted earlier, all these findings with respect to alcohol administered groups are in agreement with earlier reports separately. Taken together, a comprehensive analysis reveals that there exists sex difference in normal levels of plasma biochemical profiles. Further it is also evident that the alcohol induced changes in female rats receiving 4g of ethanol/kg b.w/day appear to be equal in male rats receiving a 2.5 fold higher dose i.e., 10g of ethanol/kg b.w/day. These results suggested that females are more sensitive to alcohol at lower doses.

Though the precise molecular mechanism(s) of these differences related to gender control in rats is not clear, the results of the present study demonstrated that differential composition and metabolic regulation as well the hormonal milieu play a vital role.

Maintenance of stable levels of blood glucose is one of the finely regulated of all the homeostatic mechanisms in which various hormones, enzymes, tissues and other factors play a role. It is well known that glucose is an essential substrate for central nervous system and all the cells of the body\(^1\). Kidneys and liver work in concert to maintain blood glucose homeostasis where any significant decrement or increment in glucose output capacity within either organ may lead to deleterious consequences associated with hypoglycemia or hyperglycemia\(^2\). Further our results indicated that in female rats chronic alcohol consumption increased blood glucose levels significantly.
Alcoholic male rats receiving 4g of ethanol/kg b.w/day did not show any change with respect to blood glucose concentration but alcohol consumption 10g ethanol/kg b.w/day increased the glucose concentration markedly. From the data of the present study it is evident that control female rats had lower blood glucose levels when compared to control males.

The effect of alcohol consumption seems to be more prominent in females. Glucose homeostasis appears to be disturbed much. Changes in all other pathological biochemical markers pointed severe damage induced by the alcohol consumption in female rats even at lower dose i.e., at 4g ethanol/ kg b.w/day.

Further overproduction of increased levels of nitrite and nitrate in plasma of alcoholic rat groups clearly indicated overproduction of NO leading to alcohol induced liver injury in females. Male rats with intake of 4g of ethanol/kg b.w/day did show modest hike in NO with no pathological signs. 10g male rats receiving ethanol/kg b.w/day and 4g female rats receiving ethanol/kg b.w/day exhibited higher levels of NO₂ and NO₃ suggesting overproduction of NO with severe pathological signs (Fig 2).

The enzyme activities of ALT, AST and γ-glutamyl transferase are considered to be a sign of alcohol abuse and generally increase in presence of alcoholism.

Ethanol administration causes significant change in metabolism of lipids and lipo-proteins leading to hyperlipidemia and development of fatty liver. Our data indicate increased levels of total phospholipids, total cholesterol with changes in lipo-proteins (decreased HDL, increased LDL, and also elevated VLDL) in both sexes. Though increments in LDL and VLDL are in agreement with earlier studies, a marked decrease in HDL is a striking observation in the present study. It is well known
that the effects of chronic alcohol consumption on hepatic lipid metabolism are complex (Table 3).

Accumulation of fat in the liver on chronic alcohol intake acts as a stimulus for the secretion of lipo-proteins into blood stream and the development of hyperlipidemia. Moreover, decreased fatty acid synthesis would increase the availability of substrate for lipo-protein synthesis. Plasma lipo-protein lipase (LPL) is an important enzyme responsible for the hydrolysis of triglycerides present in chylomicrons and VLDL. Low activity of LPL in alcohol fed rats causes accumulation of triglycerides and hydrolysis of VLDL. In general HDL, which is considered to be a beneficial protein, helps in scavenging cholesterol from extrahepatic tissues in presence of lecithin-cholesterol acyl transferase (LCAT) and brings it to liver. In present study the HDL concentration in plasma was significantly lower in rats receiving alcohol in comparison with controls in both sexes. Increased serum VLDL and LDL with a decrease in HDL concentration observed in the present study in alcoholic rats suggest cardiac risk as well hepatic dysfunction.

Elevated plasma triglyceride, cholesterol, phospholipids, as well LDL-C, VLDL-C and atherogenic index with decreased HDL-C were observed in alcohol receiving rats in the present study. In general, hyperlipidemia is a known complication of alcohol toxicity leading to cardiovascular problems and atherosclerosis.

Changes in lipoprotein cholesterol patterns in receiving female rats alcohol 4g ethanol/kg b.w/day when compared to respective controls appear to be similar to that of males 10g ethanol/kg b.w/day when compared to respective controls suggesting that females are more prone for lipid and lipoprotein abnormalities even at lower dose. In
present study the results showed that control female had lesser lipid fractions when compared to control males. However, alcohol consumption influenced lipid metabolism much causing lipid and lipoprotein abnormalities in females.

**Histopathological changes**

Size of the liver and weight of the liver in females was found to be smaller when compared to males. Probably owing to smaller size liver cells appear to be packed with compactness. Because of compactness of lobulization cells also appeared to be small in females.

When liver sections were examined histologically both the normal male and female sections appeared to be the same with lobulization. Cells are hexagonal in shape with conspicuous nuclei. Interlobular spaces are limited, but no haemorraging conditions were seen (Fig 2, 3).

Sections obtained from 4g ethanol/kg b.w/day female and 10g ethanol/kg b.w/day male showed pathological signs with cirrhosis characterized by the loss of lobulization, increased interlobular spaces with edematic vacuolization, loss of hexagonal shape of cells, atrophy of cells and nuclear necrosis (Fig 5, 6). 4g male sections did not exhibit any pathological symptoms the sections were almost similar to that of normal sections with clear cut lobulization. Though little fibrosis was shown there was no necrosis (Fig. 4).

From the above histopathological observations it is evident that the damage caused by 4g ethanol/kg b.w/day in female appears to be equal to 10g ethanol/kg b.w/day in males suggesting that female are sensitive to alcohol induced damage at
lower dose. Further 4g ethanol/kg b.w/day did not induce significant damage in male suggesting the tolerance of in male rats to alcohol at the selected dose. Further, the damaged caused in female rats consuming 4g/kg b.w/day.

4g/kg b.w ethanol induced damage in females is equal to that 10g/kg b.w ethanol induced damage in males suggesting females are more sensitive to ethanol toxicity.

Ethanol metabolism occurs mainly in the liver, a sex hormone responsive organ that presents distinct responses to both estrogens and androgens. Specific hormone responsive proteins and enzymes have been identified. Hepatocytes and non parenchymal liver cells express estrogen receptors and androgen receptors. Androgens are major determinants of sexually dimorphic aspects of hepatic function. Liver responds to estrogen by increasing the synthesis and secretion of many serum glycoproteins such as ceruloplasmin, corticosteroids binding globulin, thyroid binding globulin and testosterone estradiol binding globulin. Further estrogen is presumed to enhance the activity of resident macrophages of the liver and kupffer cells. The kupffer cells play a central role in pathogenesis of alcohol induced liver injury. Lack of androgen production of the liver from alcohol induced oxidative injury in the females appear to be susceptible for chronic alcohol toxicity.

Both humoral and cell mediated immunity are more active in women than in men and it is believed that female sex hormones regulate immune responsiveness. Liver is a site of metabolism of sex hormones and is a hormone responsive organ. Estrogen receptors and receptor pathways modulate some kupffer cell activities. Further kupffer cells from female rats are known to produce higher amounts of toxic and proinflammatory modulators among which are ROS, as compared to males. Production of tumor necrosis factor (TNF) by kupffer cells in response to
lipopolysaccharide is increased insignificantly by estrogen treatment in rodent in vitro and in vivo. Estradiol has been shown to increase the expression of CD14, the LPS receptors, on kupffer cells and the production of LPS binding protein by liver. These two elements trigger the stimulation of macrophages which are known to lead to pathogenesis. Further it is also known that androgens are involved in modulation of hepatic alcohol metabolism with the direct actions on hepatic enzyme activities by interaction of androgens with androgen receptors. The T dependent inhibitory effects on the activity of hepatic ADH representing a defense of male hepatic cell against alcohol mediated oxidative stress has been reported. Androgens exert many beneficiary effects by modulating activity of immune system. Androgens have ROS scavenger properties. Dehydroepiandrosterone (DHEA) inhibits macrophage superoxide production as well oxygen free radical scavenging properties. DHEA treated rats become more resistant to iron dependent lipid peroxidation. The biochemical basis for this action is the inhibition by DHEA on glucose-6-phosphate dehydrogenase, the initial enzyme in the pentose phosphate shunt necessary for nicotinamide adenine dinucleotide phosphate generation and superoxide anion formation.

Biophysical and biochemical changes related to membranes vs alcohol consumption

It is important to note the term membrane fluidity is often misused. It arose from a combination of spectroscopic studies, the realization that membrane can be regarded as 2 dimensional fluids, and desire to obtain a simple single physical parameter that would describe their properties. The difficulty with membrane fluidity concept is that any physical parameters chosen will be a function of spectroscopic
method implied, specifically in particular time window and the properties of the probe (shape, charge, location etc)\textsuperscript{31}.

The membrane fluidity concept also depends on the assumptions that the hydrophobic regions of the cell membrane are structurally and dynamically homogeneous, an assumption that is now under serious challenge. Thus, while it may be true to say that the bulk or average spectroscopic properties of cell membranes may not be useful in building a hypothesis for pharmacological action(s) of drug(s). Local properties pertaining to domains or the immediate environment of the protein may be very relevant\textsuperscript{27-29}.

As already pointed out membrane bilayer mobility is one of the important factors controlling membrane microviscosity, rotational mobility and flip-flop and it is well known that most important of these lateral mobility\textsuperscript{31}.

Annular fluidity obtained by pyrene excitation with fluorescence energy transfer from tryptophan (in adjacent proteins) indicated a decrease in the fluidity of the micro environment surrounding proteins. Fluorescence energy transfer from tryptophan to pyrene occurs efficiently only with the defined Forster radius (which is in range of nanometers), thus allowing for evaluation of viscosity and lateral diffusion rates of membrane domains in close proximately with membrane proteins. Membranes have a laterally heterogeneous distribution of lipids and proteins\textsuperscript{31}. Receptor proteins and enzymes are selectively segregated in to membrane microdomain called lipid rafts, as opposed to the random distribution proposed by the fluid mosaic model\textsuperscript{32}. The fatty acyl chains in phospholipids and sphingomyelins are mostly saturated which in association with raft cholesterol, contribute to decreased fluidity in the raft (annular
fluidity) regions of the membrane. Fluorescence measurements with pyrene (decreased E/M ratio) indicated decreased annular fluidity. This might be caused by either increased cross linking of membrane components by lipid peroxidation in various membranes and/or an increase in ceramide, a product of sphingomyelin degradation in erythrocyte membrane following the drug treatment. In the present study the ethanol induced overproduction of nitric oxide may have contributed for the observed lipid changes in membranes and cells. The mechanism by which NO causes pathophysiological changes with the mediation of membrane changes is still unclear. NO may cause pathophysiological changes via oxidative stress and lipid changes.

Results from the present study reveal an increase in red cell as well mitochondrial cholesterol membrane content with no change in phospholipids followed by a consequent increase in C/P ratio suggesting a decrease in membrane fluidity (Table 4). Decreased fluidity or increased rigidity results in mechanical instability leading to alternations in cellular function and/or believed to be important early steps in aetiology of atherosclerosis and coronary problems that result in chronic excessive alcohol toxicity. There was no change in fluidity in outer domain as evidenced by no significant change in E/M ratio when pyrene was employed. This indicated the permissive effect of alcoholism. It is surprising to note the increased anisotropy (γ values). Use of DPH in the study indicated decreased fluidity in hydrophobic domains of the bilayer of hepatic mitochondrial membranes of alcohol receiving rats. The unaltered lipid contents and the consequent C/P ratio as well no significant changes in E/M ratios and also DPH anisotropy values suggesting permissive or no effect of chronic alcoholism on brain mitochondria in both the genders. Probably, some compensatory arrangements to maintain cerebral mitochondrial membrane fluidity so as to maintain membrane milieu for proper
functioning might be the cause for the observed event in which the possibility of involvement of NO cannot be ruled out. Further, it is also evident from the study that hepatic mitochondria are susceptible for alcohol induced adverse changes when compared to brain mitochondria (Table 5).

Microsomal bilayers showed a small but significant increase in the contents of cholesterol and phospholipids but no significant changes in the consequent C/P ratio pointed to unaltered fluidity. Studies using fluorescent probes such as pyrene and DPH have confirmed the same. In all, it is clear that microsomal membranes appear to be tolerant to ethanol treatment in both the genders\(^5\) (Table 6).

DPH (1,6-diphenyl -1, 3, 5-hexatriene) is a well known probe for monitoring the fluidity of the native membranes by fluorescence membrane. The fluorescence polarization of DPH incorporated into natural membrane is monitored in control and alcohol receiving rat mitochondrial and microsomal membranes with DPH fluorescence polarization. Alcohol intake caused a considerable increase in \(\gamma\) values (decrease in DPH polarization in both mitochondrial and microsomal preparations examined) indicating decreased fluidity\(^6\).

While DPH partitions selectively into the membrane coat constituted by phospholipid fatty acyl chains, pyrene moves laterally and thus enabling the estimation of membrane fluidity in both regions\(^7\). Changes in C/P ratio, saturated: unsaturated fatty acid as a result of increased lipid peroxidation leads to decreased membrane fluidity\(^8\).
Oxidative stress and overproduction of nitric oxide vs alcohol consumption

Data from the present study reveal lower levels of nitrite and nitrate in different compartments viz., plasma, liver mitochondria, brain mitochondria and also in microsomes of control female rats when compared to control males (Fig 7, 8, 9, and 10). Further it is also evident from the data that alcohol consumption causes dramatic change in nitrite concentrations reflecting NO production. Though modest hike in NO concentration is reported to be beneficial, reports also revealed that overproduction of NO leads to toxicity. Zima et al.,40 and others have attributed ethanol tolerance to NO39. Excessive NO mediates the formation of more potent oxidants such as peroxynitrite (O2-+NO----->NOO) and peroxynitrite formation further enhances oxidative stress.

Calcium-dependent NOSs are responsible for the generation of NO in cardiomyocytes, endocardial endothelium, coronary endothelium, and cardiac nerves. NO serves many important physiological roles in the regulation of cardiac function including coronary vasodilation, inhibiting platelet and neutrophil adhesion and activation, modulation of cardiac contractile function, and inhibiting cardiac oxygen consumption41-42. NO is necessary for normal cardiac physiology and plays a protective role in the ischemic heart by numerous mechanisms including stimulation of soluble guanylyl cyclase to decrease of intracellular Ca2+ through activation of cGMP-dependent protein kinase, as well as termination of chain-propagating lipid radical reactions caused by oxidative stress43-44 (Fig.14). NO, through its interactions with components of the mitochondrial respiratory chain, may function as a physiological regulator of cell respiration and a modulator of the generation of reactive oxygen species by mitochondria, thereby affecting mechanisms of cell survival or death45. In higher levels, or in the presence of reactive oxygen species, NO can also exert cytotoxic
effects, potentially through the formation of peroxynitrite. The hypothesis that peroxynitrite generation contributes to myocardial and vascular dysfunction during ischemia and reperfusion (I/R), myocarditis, chronic heart failure, and various other cardiovascular pathologies has been the focus of intensive investigations during the last decade.

Overwhelming evidence has established a role for NO in essentially all major pathological processes affecting humans. But NO itself is unlikely to be the proximal toxin in these processes. It is not highly reactive and is efficiently removed by the reaction with hemoglobin in red blood cells. It takes two oxygen molecules supplied by oxyhemoglobin in red blood cells for NOS to produce each molecule of NO. Because NO diffuses slightly faster than oxygen, it will be able to diffuse back to a red blood cell. Consequently, there will always be an efficient sink to remove NO in blood perfused tissues that will prevent NO from forming significant amounts of reactive nitrogen species.
Cardiac insult
Myocardial infarction, ischemic heart disease, hemodynamic overload (hypertension, aortic stenosis, valvulopathy, cardiomyopathy, etc.)

Cardiovascular protection

Nitric oxide (NO) by activating soluble guanylate cyclase (sGC)-cGMP signal transduction pathway mediates various physiological/beneficial effects in the cardiovascular system including vasodilation, inhibition of platelet aggregation, anti-inflammatory, antiremodelling, and antiapoptotic effects. On the other hand, under pathological conditions associated with increased oxidative stress and inflammation (myocardial infarction, ischemic heart disease, myocarditis, cardiomyopathy, hypertension, etc.), NO and superoxide (O2·−) react to form peroxynitrite (ONOO−) which induces cell damage via lipid peroxidation, inactivation of enzymes and other proteins by oxidation and nitration, and also activation of stress signaling, matrix metalloproteinases (MMPs) among others (see also Table 2). Peroxynitrite also triggers the release of proapoptotic factors such as cytochrome c and apoptosis-inducing factor (AIF) from the mitochondria, which mediate caspase-dependent and -independent apoptotic death pathways. Moreover, peroxynitrite, in concert with other oxidants, causes stand breaks in DNA, activating the nuclear enzyme poly(ADP-ribose) polymerase-1 (PARP-1). Mild damage of DNA activates the DNA repair machinery. In contrast, once excessive oxidative and nitrosative stress-induced DNA damage occurs, like in various forms of myocardial reperfusion injury and heart failure, overactivated PARP initiates an energy-consuming cycle by transferring ADP-ribose units from nicotinamide adenine dinucleotide (NAD+) to nuclear proteins, resulting in rapid depletion of the intracellular NAD+ and ATP pools, slowing the rate of glycolysis and mitochondrial respiration, eventually leading to cellular dysfunction and death. Poly(ADP-ribose) glycohydrolase (PARG) degrades poly(ADP-ribose) (PAR) polymers, generating free PAR polymer and ADP-ribose. Overactivated PARP also facilitates the expression of a variety of inflammatory genes leading to increased inflammation and associated oxidative stress, thus facilitating the progression of cardiovascular dysfunction and heart failure.
However, NO can be quickly converted into a series of powerful oxidants with many biological effects by its diffusion-limited reactions with many free radicals. The major physiological gateway to produce reactive nitrogen species is most likely through the diffusion-limited reaction with superoxide. While a small flux of superoxide is inevitably produced by leakage of electrons to molecular oxygen through the autoxidation of biological molecules, there is growing recognition that cells can be activated to produce large amounts of superoxide by specific NADPH oxidases and other enzymatic sources. Because the rate of peroxynitrite formation rises 100-fold for each 10-fold increase in superoxide and NO production, the production of superoxide offers a dynamic mechanism to redirect NO from being a signaling molecule to an important component of host-defense and innate immunity.

The range of free radical reactions potentially involving NO is bewildering, leading most investigators to retreat into the relative safety of attributing the effects to terms such as "reactive nitrogen species" or more broadly "reactive nitrogen and oxygen species". But, with this comes a substantial cost in understanding the underlying mechanisms. Once significant amounts of peroxynitrite are produced in a cell, it will produce a shower of other reactive nitrogen species. Peroxynitrite is likely to be the major source of both nitrogen dioxide and possibly nitrite in vivo. When NO is produced more rapidly than superoxide, the excess NO will be consumed to produce a variety of nitroso species, which implies that a modest stimulation of superoxide formation will yield nitrosative rather than nitrative stress.

The broad actions that can be attributed to peroxynitrite in inflammation, cardiovascular disease, neurodegeneration, diabetes, and other pathologies have been reviewed here in some detail. Multiple lines of evidence support the pathophysiological
role of peroxynitrite. Its footprints are detectable by using various methods in virtually all diseases both in humans and animals. Potent enzymatic scavenging systems to detoxify peroxynitrite are found in pathogenic microorganisms. Selective scavengers of peroxynitrite exert beneficial effects in various animal models of disease, and improvements of many pathophysiological conditions in general are associated with decreased target tissue nitrotyrosine formation. Compounds such as urate and many polyphenolics are protective by acting as alternative targets for tyrosine nitration. Many other therapeutic approaches, such as iNOS inhibitors, superoxide scavengers, NADPH oxidase inhibitors, and broadly effective antioxidants (e.g., vitamin E, ascorbate, melatonin, etc.), may be in part protective by preventing the formation of peroxynitrite or repairing damage initiated by it. Various commonly used medications currently used to treat human disease (e.g., ACE inhibitors, carvediol, etc.) also decrease nitrotyrosine formation in diseased tissues. Peroxynitrite may play an important role in modulating vascular injury as well as proinflammatory responses.

Human and experimental animal studies on alcoholics direct effect on the heart in the intact organism are complicated by alcohols indirect effects on the other organ systems and molecules that modulate heart function. Alcohol is known to induce changes in the levels of catecholamine. Earlier studies suggested that alcohol interferes with contractile function of the heart muscle. Chronic excessive alcohol consumption has been associated with a variety of cardiovascular diseases ranging from hypertension and stroke, dilated cardiomyopathy and hypertension to heart failure and sudden death. Though moderate alcohol consumption appears to reduce the risk of CHD, several reports clearly suggested that alcohol abuse leads to coronary risk and other cardiovascular diseases.
Increased formation of MDA in various compartment and organelles observed in the present study is an indicative of enhanced lipid peroxidation and also a marker of oxidative stress. This finding is in agreement with some earlier studies. Lipid peroxidation is a chain reaction process in which each step is involved in providing a continuous supply of free radicals that initiate further peroxidation and whole process involves in initiation, propagation and termination phases each of which is influenced by a wide range of factors. Peroxidation of lipids in biological systems has attracted the attention of the most in recent years as it leads to damage of tissues in vivo, where it may be cause of atherosclerosis and various complications associated with alcoholism. Tissues isolated from animals are shown to contain lipid peroxides in low concentration and further produce more peroxides during cellular damage as a result of pathological conditions. Peroxidation causes considerable changes in the structural organization and functions of cell membranes and makes the membrane leaky. Furthermore, a number of pathological phenomena such as increased membrane rigidity, in case of erythrocyte membrane, decreased cellular deformability, reduced erythrocyte survival and lipid fluidity were reported. Whether lipid peroxidation occurs as a consequence of free radical activity or vice versa is yet to be confirmed. Increased lipid peroxidation observed in the present study in plasma, erythrocyte, erythrocyte membrane and liver of alcoholic rats is in agreement with earlier studies.

Available literature suggested the generation of oxygen and ethanol derived free radicals at microsomal level by intervention of ethanol inducible cytochrome P450 isoform (CYP2E1). Further, ethanol linked enhancement in free radical generation through the cytosolic xanthine and/or aldehyde oxidases as well through mitochondrial respiratory chain resulting in oxidative stress leading to lipid peroxidation. Reactive
Oxygen species (ROS) are small, highly reactive, oxygen containing molecules that can react with and damage complex cellular molecules, particularly in the liver. Ethanol metabolism is directly involved in not only the production of reactive oxygen species (ROS), but also related in the formation of an environment favorable to oxidative stress such as hypoxia, endotoxemia, and cytokine release. Further, alcohol can alter the levels of certain metals in the body, thereby facilitating ROS production. Finally, alcohol reduces the levels of antioxidants which can eliminate ROS. Following ethanol intoxication, the balance between pro-oxidants and antioxidants is distributed to such an extent that it results in the oxidative damage of biomolecules, such as fats, proteins, or DNA and finally leading to cell injury. ROS production and oxidative stress in liver cells play a central role in the development of alcoholic liver disease. Oxidative stress provides a mechanism by which alcohol intake may be linked to hepatic inflammations and fibrosis. Ethanol induced oxidative stress is important in stimulating immune reactions towards both liver allo-and self-antigen.

**Antioxidant status vs alcohol consumption**

Glutathione is a tripeptide present in almost all the cells and plays an important role in metabolism, transport and cellular production against oxidation by free radicals and reactive oxygen intermediates. The levels of these reactive oxygen species are controlled by antioxidant enzymes viz., glutathione reductase, catalyzing the reduction of GSSG to GSH utilizing NADPH generated in HMP shunt pathway, glutathione peroxidase catalyzing the reduction of hydrogen peroxide and organic hydroperoxides using GSH, superoxide dismutase catalyzes the reduction of superoxide radical to hydrogen peroxide and catalase catalyzing the reduction of hydrogen peroxide to water and glutathione-S-transferase is multifunctional protein.
catalyzing the conjugation between GSH with a wide variety of secondary substrates and play, an important role in the detoxification of xenobiotics.

The endogenous glutathione-glutathione peroxidase system and catalase are important antioxidants and cytoprotective machinery in the hepatocytes exposed to ethanol. Glutathione reductase is concerned with the maintenance of cellular level of reduced GSH. Glutathione reductase and catalase activities are increased as a consequence of ethanol exposure. Chronic ethanol consumption induces alternations in the glutathione/glutathione peroxidase antioxidant system, which might promote oxidative modification of liver mitochondrial proteins, leading to the adverse effects on the liver. The glutathione-S-transferase (GSTs) are a group of polymorphic enzymes that are important in the protection against oxidative stress.

In present study decreased activities of catalase, superoxide dismutase, glutathione peroxidase and the content of reduced glutathione in red cell, mitochondria (liver and brain) and liver microsomes of alcohol fed male and female rats indicated decreased antioxidant mechanism as well defense status in chronic alcoholic rats when compared to controls making them (alcoholic rats) susceptible to free radical induced diseases (Tables 8, 9, 10 and 11). These findings are in agreement with earlier reports. Increased oxidative stress and decreased antioxidant mechanisms and overproduction of NO together lead to alcoholic damage in the present study.

Result of the study show that activities of mitochondrial (liver and brain) ATPases (Na+, K+ and Mg2+) and monoaminoxidase were stimulated in alcohol receiving rats when compared to controls. (Tables 12 and 13). In addition to increase in
activity of ATPases, a hike in cytochrome P 450 is noticed in the present study suggesting the influence of alcoholism (Table 14).

Appearance of higher sulphydryl groups and lower carbonyl group in females than males indicated differential metabolic events which were gender dependent. Alcohol consumption caused increased contents of both sulphydryl and carbonyl contents in both sexes (Fig.11, 12 and 13). It is well known that thiol containing proteins appear to be targets of free radicals causing damage to cellular proteins associated with chronic ethanol intake. Oxidative modifications of mitochondrial and microsomal proteins thiols due to chronic alcohol consumption leads to altered thiol redox status. This in turn comprises the mitochondrial membrane polarization which is essential for ATP synthesis by mitochondria and dysfunction of microsomes. Further increased cytosolic and mitochondrial carbonyls as well accumulation of damaged proteins playing a pathogenic role in experimental alcoholic liver disease were reported. Moreover various lipid peroxidation products bind to proteins forming stable adducts stimulating fibrogenesis and histological damages in alcoholic liver disease. In addition HER, HNE and other radicals are involved in modifying cellular proteins. Though chronic ethanol feeding was reported to cause upregulation of the enzyme at m-RNA level and over expression of Mn-SOD preventing ethanol induced liver damage in rats, in present study we found diminished/decrease activities of SOD as well other defense enzymes and GSH in ethanol receiving rats. Probably due to loss of adaptive response of defense mechanisms might have led to alcoholic damage observed in the present study as suggested by Koch et al. (1994). Bailey et al., have observed greater increase in protein content in carbonyl content which was attributed to the development of pathogenesis related to alcoholism. Studies of Venkataraman et al., using alcoholic rats demonstrated that alternations in protein thiols have biological
relevance and contribute to the pathologies associated with several disease states such as alcoholic liver disease.

To sum up differential metabolic regulation, differential composition and nervous control mechanisms, lack of androgen protection and estrogen mediation, differential hormonal milieu and dysfunction of nitric oxide metabolism are responsible for the observed greater sensitivity of females to alcohol and alcohol toxicity.
References


