REVIEW OF LITERATURE
Alcohol and tobacco are the most widely used psychoactive substances throughout the world and are often used together. In view of strong relation between alcohol use and smoking, combined use of alcohol and tobacco, present study is an attempt to understand the biochemical events and mechanisms associated with the joint exposure of alcohol and cigarette smoking in human subjects.

Alcohol-metabolism and effects

Alcohol is often consumed by people for mood lifting and recreational purposes throughout the world. Though moderate alcohol consumption has some benefits such as relaxation, protective effects on heart and beneficial modulation of certain diseases, moderate consumption often leads to addiction and finally results in excessive and chronic alcoholism with several ill effects (Goldberg et al., 1999; Gaziano et al., 2000). Chronic and excessive consumption of alcohol is dangerous leading to alcoholic liver disease, chronic pancreatitis, cardiovascular diseases, brain damage and neurological disorders (Lucas et al., 2005; Xu et al., 2005). World Health Organization (2004) reports revealed that 63 diseases are directly and many are indirectly linked to alcoholism (World Health Organization, 2004). Despite the fact that alcohol is responsible for many adverse socio-economic and health consequences in many countries, people have been consuming alcohol in excess (Xu et al., 2005). Moreover, new drinkers are added to the list every year including teenaged boys and girls with increased worldwide sales of alcohol (Lieber, 2000). A lot of work done on alcoholism reveal much information and various facts related to metabolism, pathologies, biochemical mechanisms, tolerance and addiction and related mechanism in humans and animals and facilitated us to understand new concepts, theories and several pathways successfully (Seitz et al., 2005). Our earlier studies also revealed several facts related to alcohol induced biochemical changes in alcoholics alone (Paramahamsa et al., 2004), alcoholic diabetes (Paramahamsa et al., 2002) as well in heavy and moderate alcoholics as well the role of nitric oxide on alcohol induced events (Kavitha et al., 2008).
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. While small amounts of alcohol acts as a drug producing euphoria for some people, 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 (Insel et al., 2004). 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) (Insel et al., 2004).

Consumed alcohol requires no digestion prior to absorption and absorption begins immediately in the mouth and esophagus where small quantities enter the blood stream. Although alcohol absorption continues in the stomach, the small intestine efficiently absorbs most of the alcohol a person consumes (Ramakrishnan, 1983). Unlike other anaesthetics, alcohol is consumed in relatively large quantities for longer periods (Lieber, 1991). Blood alcohol levels (BAL) 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 (Hoek and Taraschi, 1988). When equal amounts of alcohol is consumed by man and woman, blood alcohol concentration in women rapidly rises to the maximum when compared to men in general (Hoeksema, 2004). Carbonation hastens absorption, whereas food delays it. Alcohol from blood is cleared rapidly as little is lost through lungs by diffusion and its further passage to all other tissues through capillary net work with which it comes in contact till it reaches equilibrium between blood and tissues except adipose tissue (Narayana Reddy, 1994). About 80% of alcohol absorbed is oxidized in the liver and the remaining 10% is excreted in breath, urine, sweat, saliva, milk, tears and faeces (Ramakrishnan, 1983; Lieber, 1991; Narayana Reddy, 1994). However in the digestive tract, mainly in the stomach alcohol diffuses into gut cells and travels via portal vein directly to the liver where most alcohol metabolism takes place. On the other hand, alcohol is subjected to renal clearance (Seitz and Oneta, 1998). The body works extra hard to get rid of it and quickly metabolizes and it removes
from blood. If blood alcohol levels reach 0.04% (1 or 2 drinks) leads to mild, pleasant changes in mood and release of inhibitions. With more than 2 drinks, blood alcohol levels rise leading to impairment of coordination, judgment, reaction time and vision. People with blood alcohol levels 0.05-0.08% are not allowed to drive motor vehicles in United States of America and Canada (NIAAA, 2001). Consumed alcohol enters every organ system through the blood stream and affects all the tissues especially brain, heart, liver and gastro-intestinal tract as shown in Figure 1.

**Figure 1** Multiple pathological effects of alcohol toxicity affecting several body organs
As alcohol through circulation reaches the brain immediately after consumption, the alcohol concentration rises in different parts of the brain which are affected.

**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.

Figure 2 Pathological effects of alcohol on brain
The liver selectively metabolizes alcohol and there exists alternative pathways to handle excess consumption (Swift and Davidson, 1998). Mitochondrial alcohol dehydrogenase, a zinc containing enzyme converts alcohol to acetaldehyde and aldehyde dehydrogenase quickly and effectively converts acetaldehyde to acetate. When large amounts of alcohol prevail, the microsomal ethanol oxidizing system (MEOS) operates at a faster speed to process alcohol quickly and converts it to acetaldehyde. Hence this route is an overflow pathway (Insel et al., 2004). 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⁺.

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

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

Acetaldehyde is then oxidized to acetic acid 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 (Elkeles and Tavil, 1983). 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 (Swift and Davidson, 1998). Recent studies suggested that acetaldehyde directly participates in the pathogenesis of alcoholism (Ramakrishnan, 1983; Elkeles and Tavil 1983; Niemela, 2007). Chronic administration of acetaldehyde during 3 weeks induced metabolic tolerance to ethanol. Alcohol induced changes in blood, liver, muscle, brain and heart were reported. Changes in ratio of activities of plasma enzymes such as
Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) with an increase was noticed in alcoholics. Hike in plasma γ-glutamyl transferase (γGT) serves as indicative of alcohol intake.

Liver is a hormone sensitive organ playing a key role in maintaining the internal environment (homeostatic balance) in higher organisms and is also a target organ for alcohol where alcohol is broken down to a number of potentially dangerous byproducts such as acetaldehyde and highly reactive free radicals (Colantoni et al., 2000). Alcoholic liver damage is well known and occurring in three phases - fatty liver, alcoholic hepatitis and alcoholic cirrhosis (French et al., 1993). Hepatic mitochondria is a chief target organelle of ethanol intoxication. There have been several studies reporting alcohol induced mitochondrial damage and also concerning the effects of ethanol on fluidity of membranes, metabolism as well pathogenesis (Chen et al., 2000; Kanbagli et al., 2002). Alcohol induced structural as well functional damage of brain at cellular level leading to several neurological complications is reported-Kornskoff’s psychosis (Bisahi and Bozzetti, 1986), Alcoholic cerebellar degeneration (Victor et al., 1989), Alcoholic Dementia (Walker et al., 1981), Central pontine myelinolysis (Charness and Diamond, 1984), Alcoholic neuropathy (Victor and Adams, 1961), Alcoholic myopathy (Urbano-Marquez et al., 1989). Alcohol induced degenerative disease of heart muscle, cardiomyopathy, characterized by depressed cardiac output has been reported. Involvement of nitric oxide in several alcohol induced events related to every organ has been reported (Poschl and Seitz, 2004; Yuan et al., 2006). Though heavy alcohol drinking has an unfavourable effects on serum lipids and lipoproteins, several epidemiological investigations have indicated that alcohol consumption exerts a dose dependent promotion or deceleration of atherogenesis. Moderate use of alcohol appears to raise HDL-C and triglycerides and lower LDL-C levels, and thereby lowering atherosclerosis risk (Hines and Rimm, 2001).

Cigarette smoking – effects and composition

Cigarette smoking is a reprehensible habit that has spread all over the world and a considerable amount of research over the past 50 years reveal that smoking alone is the
cause for variety of serious diseases such as CVD, CHD, lung cancer, emphysema, chronic bronchitis (Esterbauer et al., 1989; Berard et al., 2002; Sood et al., 2003). There has been strong relation between cigarette smoking and alcoholism. Evaluation of the effects of smoking on health would not be proper if beneficiary effects of smoking are not taken into consideration. Due to certain specific constituents such as nicotine cigarette smoke exerts psychoactive, euphoric, reinforcing and produces tolerance to cope with stress (Michael et al., 2004). Other useful effects include weight control, mood control and relief of tobacco withdrawal symptoms (Epping-Jordan et al., 1998). Besides the role of smoking in maintenance of good intestinal tone and bowel habits are well documented (Schneider and Ivy, 1939). Moreover antiobesity effects upon reduced hunger and a possible elevation in blood sugar are reported (Brozek and Keys, 1957). Unlike alcohol and other drugs, cigarette smoking or its constituent nicotine does not impair performance in judgment, cognition or motor behaviour. In addition it may slightly improve performance and health of people to cope with daily stress (Michael et al., 2004). It is generally asserted that smokers can take self decisions effectively, quickly compared to non-smokers and the decision taken is also often said to be rapid, apt, bold and also effective to get good results (Breslau et al., 1993; Kassel et al., 1994). Recently some more beneficiary effects of smoking/nicotine are coming into light (Elizabeth et al., 2005). Cigarette smoke consists of nicotine, nitric oxide, some aldehydes and other components along with oxidant free radicals (Church and Pryor, 1985). While some of the former constituents exert antioxidant properties and also appear to be neuroprotective, others cause damage (Rustemeier et al., 2002). Many evidences suggest that cigarette smoking has neuroprotective effects by exerting certain desirable effects in Parkinson’s disease and Alzheimer’s disease by activating AchR and also by inhibiting MAO activity (Berlin et al., 2000). Besides cigarette that smokers are less susceptible for Alzheimer’s disease and Parkinson’s disease (Fratiglioni and Wang, 2000). Various studies revealed that cigarette smoking may be neuroprotective, activate NAcHR (neuronal nicotine acetylcholine receptors) and inhibit monoamino oxidase in glial cells (Elizabeth et al., 2005). Other studies also support that upregulation of nicotinic receptors in the basal ganglia can provide partial protection against dopaminergic neurodegenerative processes (Le Novere et al., 1996). In humans, cigarette smoking or administration of nicotine can activate
NACchRs leading to an increase in striatal dopaminergic activity effects that correlate with attenuation of tremor, rigidity and brodichinasia and improved cognitive functions in PD patients (Kelton et al., 2002; Quik and Kulak, 2002).

A burning cigarette is a complex system in which many types of chemical reactions and physical processes occur in parallel. During combustion oxygen reacts with carbonised tobacco producing simple gases such as CO₂ and CO. Temperatures between 700 and 950 °C are generated during a puff and are down streamed where temperatures are in the range of 200 to 600 °C and oxygen levels are relatively low. About a third of the smoke constituents including nicotine distilled out of tobacco in this region. Lower molecular weight hydrocarbons, n-alkanes, n-alkenes, benzene, alkylbenzens and several other constituents reside in phase of the smoke. More than 75 monocyclic aromatic hydrocarbons such as a benzene and toluene are formed by the pyrolysis of amino acids, fatty acids, cinnamic acid, sugars and paraffins, precursors with an aromatic of cyclohexane ring, and pyrosynthesis from primary hydrocarbon radicals. Tobacco smoke also contains tar which is actually a composite of thousands of different substances (Geiss and Kotzias, 2007).

The chemical composition of cigarette smoke is complex, with about 4000 known active chemicals and more than 1,00,000 unknown constituents (ocular, gas phase) of which more than 40 chemicals are shown to be carcinogenic, and many others are deleterious to cardiovascular and pulmonary systems and other organs (Frei et al., 1991; Sood et al., 2003). Among these nicotine, tars, nitrosamines, polycyclic aromatic hydrocarbons, hydrogen cyanide formaldehyde and carbon monoxide are well known constituents of cigarette smoking (Church and Pryor, 1985). Besides many free radical species, aldehydes, peroxides, epoxides, nitrogen oxides, peroxyradicals and other prooxidants exist in gas phase (Pryor et al., 1983). Although nicotine is the addictive component of cigarette smoke, it should be recognized that effects of cigarette smoking are not equivalent to that of nicotine, as nicotine is one of the several thousand components of cigarette smoke (Tonnessen et al., 2000). During the blending and processing of tobacco humectants such as glycerin and propylene glycol are added to increase the moisture holding capacity of tobacco to aid in processing while flavor in
processing while flavor ingredients (non-volatile aromatic materials like menthol and also foods such as chocolate, cocoa and spices such as vanilla nut mug ginger) are used to enhance flavor of tobacco smoke (Carmines et al., 2005). Studies conducted by Carmines et al. (2005) revealed the presence of various smoke constituents in different concentrations in normal cigarettes and cigarettes containing licorice extracts in TPM (total particulate matter).

From earlier reports it is clear that cigarette smoke does contain numerous components (Witschi et al., 1997; Yuan et al., 2007). Cigarette smoke constituents can be categorized into two (i) The tar component of cigarette smoke (ii) Gas components of cigarette smoke (Keatings et al., 1996). Tar components of cigarette smoke contain an estimated $10^{18}$ spins/gram tar. The gas phase consists of as many as $10^{15}$ organic radical per puff (Repine et al., 1997; Solberg et al., 1998; Diken et al., 2001; Bruno et al., 2005).

Cigarette smoke contains polycyclic aromatic hydrocarbon benzo-pyrene and tobacco specific nitrosamine NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1butanone) which were demonstrated to be potent carcinogens causing lung cancer in mice, rats, hamsters and also humans. Besides nicotine and carbon monoxide are present in particulate phase and gas phase containing two tobacco nitrosamines NNK and NNN bipyridyl aminobiphenyl benzoquinone and several other constituents such as naphthalene phenanethrene anthracene and pyrene chrysene and some other polycyclic hydrocarbons which even enter the human systems through filtered smoke. It is possible that inhalation of benzo(a)pyrene, NNN or NNK delivers higher concentrations or larger amounts of carcinogens directly to putative target cells in the respiratory tract than does systemic administration. Gas phase contains several carcinogens such as benzene, formaldehyde, butadiene, N-nitrosodimethylamine and N-nitrosodiethylamine and nitrogen oxides. Further nitrogendioxide and nitrogen oxides of gas phase are capable of forming free radicals (Witschi et al., 1997). Bombick et al. (1997) reported that the removal of carbonyls and other volatiles decrease the cytotoxicity of cigarette smoke (carcinogenic potential of the gas phase of environmental tobacco smoke).
Biochemical studies on cigarette smoking, Diseases and risk associated with smoking

Personality and behavioral studies have suggested why some people are more likely to smoke, and what smokers perceive that they derive from smoking tobacco (Bergen and Caporaso, 1999). Addiction to nicotine has been established as the psychopharmacologic mechanism that maintains cigarette smoking behaviour (Center for Health Promotion and Education, 1988). Smokers often increase smoking intensity, smoking rate or inhalation to maintain levels of nicotine as measured by plasma levels of nicotine in both ad libitum and laboratory smoking settings (Benowitz et al., 1982; 1983; 1986a). Certain studies indicated that cigarette smoke inhalation paradoxically increased NO concentration in plasma and enhanced vascular dilation which is possibly due to exogenous NO contained in cigarette smoke (Swami et al., 2006; Cooper and Magwere, 2008). Tsuchiya et al. (2002) have observed that smoking a single cigarette temporarily decreased nitrate, nitrite and serum antioxidant concentrations in plasma and they attributed these transient changes to coronary vasoconstriction which is routinely observed after smoking. Vleeming et al. (2002) opined that NO may contribute to the development of cigarette smoking and nicotine addiction since (i) inhaled NO may facilitate increased nicotine absorption (ii) NO released through nicotine reduces symptoms of stress, and (iii) NO released endogenously by nicotine increases post synaptic dopamine levels. (iv) NOS inhibitors attenuate symptoms of nicotine abstinence syndrome. Chronic obstructive pulmonary disease (COPD) is a costly health problem and evidences suggest that oxidative stress of smoking contributes to COPD. Antioxidant therapy seems to be beneficial in COPD (Repine et al., 1997; Das, 2003). Considerable evidence now links COPD with smoking induced oxidative stress. COPD is an obstructive airway disorder characterized by a slowly progressive irreversible decrease in FEV1; FEV1 decreases are caused by a narrowing of airway lumen diameters that develop as a result of varying perturbations in both airway and interstitial lung tissue. Airway abnormalities consist of increased wall thickening, intra luminal mucous accumulation, smooth muscle hypertrophy and small air way lining fluid changes. Additional early lesions include inflammatory cell infiltration and globet cell metaplasia (Repine et al., 1997). Chronic bronchitis and chronic emphysema are two types of COPD
and many risk factors such as cigarette smoke, air pollution to chemicals, heredity and infectious and allergy conditions are implicated in the development of COPD (American Thoracic Society Task Force Report, 1995; Das, 2003). Cigarette smoking is known to decrease surfactant activity of branchioalveolar lavage fluid (Balint, 1971). Chronic smoking resulted in irregular expansion of alveolar spaces and hypertrophy type II cells in rats (Le Mesurier et al., 1981). Studies revealed that cigarette smoking inhalation causes decreases in the level of lung surfactant phosphotidyl choline (Subramaniam et al., 1995) while others reported there was no difference in lung lavage phospholipids concentration between smokers and non-smokers (Mancini et al., 1993). Probably the heterogeneous nature of cigarette smoke might be responsible for observed discrepancy and this finding indicated that the effects of cigarette smoke on surfactant and surfactant producing cells are more complex (Haagsman and Van Golde, 1995). Since the secretion of surfactant is mediated by the action of catecholamines on specific beta adrenoreceptors in lungs and desensitization of adrenoreceptors in alveolar type II cells, it explains why smoke exposure will lower the surfactant level in branchial preps (Whitsett et al., 1981; Mukharjee and Das, 1992). Reports reveal that direct and passive cigarette smoke exposure to guinea pigs causes a significant decrease in the level of a 36 Kda Ca^{2+} dependent phospholipids binding protein (PLBP) in alveolar type II cells and lung lavage (Das et al., 1992). Wang et al. (2001) reported that volatile compounds of cigarette smoke extracts of acetaldehyde and acroleine were able to inhibit human airway epithelial cell chemotaxis, proliferation and concentration of three dimensional collagen gels, a model of extra cellular matrix remodeling. Non-volatile compounds of cigarette smoke extract also inhibited chemotaxis. Cigarette smoking contributes to architectural disruption present in the airway in COPD (Fig 3). Numerous processes increase lung oxidative stress and contribute to a variety of abnormalities that contribute to COPD. Enough evidence suggests that smoking affects asthma adversely. Active smoking aggravates the problem and causes longitudinal changes in lung function and asthma related mortality (Ulrik and Lange, 2001). Parental smoking maternal smoking and ETS exposure in patients with established asthma is associated with more severe symptoms and also with lower quality of life reduced lung function and increased health care utilization for asthma including hospital admission (Weitzman et al., 1990; Das, 2003).
Figure 3 Cigarette smoke, oxidative stress, and COPD.
ETS affects asthmatic children by impairing pulmonary function (Muarry and Morrison, 1986). When lung cancer cigarette components strongly damage proteins, lipids and DNA of the cells and defense machinery will be badly affected causing generation of toxic oxygen species and reactive metabolites generated by pulmonary metabolism of foreign compounds (Jenkeinson et al., 1983). Evidences clearly suggest that smoking is responsible for the causation of female lung cancer, and several other cancers, including squamous cell carcinoma and adenocarcinoma (Rachtan, 2002; Das, 2003). The rates of lung cancer in American men have greatly exceeded Japanese men as American manufactured cigarettes contain higher concentration of tobacco specifically nitrosamines, while much wider use of activated charcoal in the filters of Japanese than in American cigarettes (Stellman et al., 2001).

Now literature is accumulating to show smoking as an important and crucial cause for the development of certain major ocular diseases such as chronic irritable eye cataract, retinal vascular disorders, tobacco amblyopia, histic optic neuropathy, thyroid eye disease, diabetic retinopathy, open angle glaucoma malignancy and age related macular degeneration (Hesker, 1995). Besides, many other ocular problems appear to be aggrevated in smokers. Smoking induces cataract formation by imposing an oxidative challenge thus contributing to the depletion of endogenous antioxidant pool. It is well known that oxidative damage plays a major role in cataract genesis. Further, tobacco smoke contains a large amount of heavy metals like cadmium, lead, and copper which accumulate in lens and exert further toxicity. Smoking is also a risk factor for retinal artery occlusion. Smoking affects choroidal blood flow in the eye and promotes ischemia, hypoxia and microinfarctions and thereby increasing the susceptibility of the macula to degenerative changes. All these may lead to age related macular degeneration (ARMD) (Sood et al., 2003).

Smoking has been implicated in the pathogenesis of various cardiovascular diseases including myocardial infarction, coronary heart disease, type II diabetes, hypertension and abnormal cardiopulmonary function. Majority literature related to smoking is on the adverse effects of chronic smoking in humans (Cresanta, 1983; Repine et al., 1997; Sood et al., 2003; Hatsukami et al., 2005). Cigarette smoke is a rich source
of oxidant free radicals and a puff of cigarette smoke is known to contain more than \(10^{18}\) spins/gram of tar, in tar phase (Repine et al., 1997). The inhaled cigarette smoke constituents (both gas and particulate constituents) interact with various plasma constituents, other cellular and membrane constituents especially lipids, proteins, nucleic acids resulting in the release of more number of free radicals in an amplified way causing a broad spectrum of effects, tissue damage and various diseases including cancers which can be grouped into cardiovascular, pulmonary, neurological disorders and miscellaneous type (Cresanta, 1983; Nair et al., 1988; Repine et al., 1997; Sood et al., 2003; Hatsukami et al., 2005). Free radicals inhaled with smoke and endogenously generated reactive oxygen derivatives enter into circulation and modulate antioxidant enzymes of blood and thereby cause cardiovascular problems (Tsuchiya et al., 2002). Though some controversy prevails over the effects of smoking on antioxidant enzyme activities it is clear that smoking is associated with disturbances by lowering antioxidant status (Diken et al., 2001; Das, 2003). Gladstone et al. (1987) reported that smoking impairs the antioxidant activity in the smokers serum. Strain et al. (1989) reported an elevation in the level of hemoglobin and ceruloplasmin but no significant effect on the antioxidant enzyme activities in the blood of smokers. Diken et al. (2001) shown changes in the enzymatic and non-enzymatic antioxidant defense systems (decreased antioxidant status) of elderly smokers and attributed the same to oxidative stress caused by cigarette smoking. Surprisingly, Toth et al. (1986) hypothesized that the increased antioxidant activities and protective abilities of erythrocytes in cigarette smokers compared with erythrocytes of non-smokers. McGowan and Hanley (1988) have found high levels of iron and ferritin in alveolar macrophages of smokers and it is possible that changes in the iron metabolism with smoking may lead to increased availability of iron for oxidant reactions and result in impaired antioxidant activity of smokers serum. A study of Belgium population revealed that low levels of serum bilirubin in smokers may be associated with lower antioxidant activity. Durak et al. (1999) reported that cigarette smoke increased oxidants in erythrocytes and supplementation of antioxidants to smokers may be beneficial to decrease cellular oxidation damages. Cigarette smoking enhanced erythrocyte lipid peroxidation and possible breakdown of antioxidant status of cigarette smokers. The only possible way to prevent and reduce adverse effects of smoking is through increasing
antioxidant status. This is possible only through consumption of phytonutrients alone. While passing through different body parts and systems, certain toxic components of cigarette smoke may be neutralized by the antioxidants present in respective tissues and in the blood. Therefore blood serves as a vehicle of cigarette smoke. Smoke is known to affect substantially several hemostatic factors leading to ischemic heart disease and the effects of smoking on hemostatic system remain for many years after giving up (Yarnell et al., 1987).

Balkayal et al. (2005) demonstrated that alcohol and smoking affected various blood variables in female and male mice with more severe effect when combined than alone. Zafer et al. (2003) have shown that smoking decreases erythrocyte count and hemoglobin level and increases leukocyte in general. Sharma et al. (2005) observed that young asymptomatic male smokers tend to have hypertension, dislipidemia and increased production of free oxygen radicals perhaps by attenuation of oxidative stress by cigarette smoking and the subjects were set to be prone for premature coronary artery disease. Various studies repeatedly confirmed that cigarette smoking increases heart rate pulse and blood pressure, cardiac output, stroke volume, velocity of contraction, myocardial contraction force and myocardial oxygen consumption, development of arrhythmia and alteration of electrocardiographic and ballisto cardiographic patterns (Sharma et al., 2005). Environmental tobacco smoke (ETS) represents a major risk factor for the generation of the diseases of the cardiovascular system. Endothelial cells of blood vessels are damaged as early as during the first month of life of passive smoking. Children with these defects can be detected during the 1st decade of life. ETS over a period of more than ten years changes the intima/media ratio by enhancing the thickness of the vessel wall. Furthermore even at young age, cigarette smoking is associated with significant detrimental effects on cardiopulmonary function and exercise tolerance (Haustein, 2001).

**Combined use of alcohol and cigarette smoke**

Cigarette smoking is common among persons with alcohol dependence or abuse with as many as 85-95% of persons who are alcohol dependent also being smokers. This combined exposure to both tobacco smoke and alcohol results in major health
consequences including additive risks for some diseases such as head and neck cancers, cardiovascular problems and many other problems (Romberger and Grant, 2004). Though nicotine appears to be the chief constituent of cigarette smoke, many other known and unknown toxic components of the smoke interact directly and indirectly exert several effects. Ribeiro-Carvalho et al. (2008) demonstrated that the central cholinergic system in mice is a site at which nicotine and ethanol interact and this interaction was thought to be associated with tobacco and alcohol consumption. The combined use of alcohol and cigarettes smoke during pregnancy by women was reported to increase the risk of low birth weight in a South Africa (Jackson et al., 2007). In view of this and adverse effects, pregnant women are cause and to reduce or prevent the use of both substances during pregnancy as most of the women who drink during pregnancy also smoke cigarettes (Odendaal et al., 2008). Reports also reveal that there is nicotine dependence as comorbidity of alcohol dependence. Further Diehl and Scherbaum (2008) stated the possible biological causes for this high comorbidity are 1) an additive rewarding affect by combined consumption, 2) substance interaction with an impact on receptor activation and metabolism which results in reduction of adverse acute alcohol effects, and 3) a combined genetic disposition for both addictions. Abreu-Villaca et al. (2007) reported that the combined use of nicotine (cigarette smoking) and ethanol (use of alcoholic beverages) resulted in detrimental effects on memory and learning. On the other hand, the combined acute effects of red wine consumption and cigarette smoking post prandily indicated an additional favorable effect of red wine. Antioxidant substances in red wine counteracted the smoking induced increase in peripheral systolic blood pressure (Papamichael et al., 2006). Serotonin (5-HT) is a biogenic amine synthesized in the central nervous system and modulates a variety of behavioral functions including the regulation of sleep, appetite, nociception, mood, stress and sexual behavior. Serotonergic dysfunction is implicated in various types of psychopathological conditions, such as antisocial personality disorder, alcoholism, depression with suicidality, antisocial behavior with aggression, obsessive-convulsive syndromes, psychosis, eating disorders, substance abuse and schizophrenia (Pivac et al., 2004). Serotonin is also reported to be synthesized by intestinal enterochromaffin cells. Serotonin is actively incorporated into platelets and stored in platelet dense granules (Culafic et al., 2007).
Both alcohol and smoking affect the physicochemical properties as well the functions of platelet membrane separately in alcoholics and smokers respectively. The combined use of alcohol and cigarette smoke has not been investigated thoroughly. Several biochemical components of platelet serve as biological markers in evaluating and confirming various health disorders including cardiovascular, neurodegenerative and psychiatric diseases. Platelet serotonin, MAO-B and as well membrane fluidity are the established indices used for health and disease. Platelets play a major role in both hemostasis, the arrest of blood flow upon serving of a blood vessel and arterial thrombosis, the formation of thrombi (masses of platelets, fibrin and other blood cells) at sights of vessel injury, and on ruptured erythroscleortic plagues. Platelet membrane has many receptors for specific ligand which induce intra and ultimately interplatelet signaling leading to activation and aggregation. Series of invaginations of plasma membrane tunneling throughout cytoplasm, different membrane bound organelles or granules containing aggregating agents, adhesive proteins and hydrolytic enzymes make the platelets unique. Actually platelets surface is a critical determinant of the normal and pathological processes. Like all other membranes the surface of the membrane is composed of lipid and proteins. There have been multiple reports that platelet MAO activity is decreased in alcohol dependent subjects compare to controls (Wiberg et al., 1977; Oreland et al., 1985; Faraj et al., 1987; Pandey et al., 1988). Both short and long term alcohol exposure affect the serotonin receptors that convert the chemical signal produced by serotonin into functional changes in the signal receiving cell. Drugs that act on these receptors alter alcohol consumption in both humans and animals. Serotonin, along with other neurotransmitters, also may contribute to alcohol intoxicating and rewarding effects, and abnormalities in the brains. Serotonin system appears to play an important role in the brain processes underlying alcohol abuse. Serotonin mediated neuronal responses to alcohol may arise from interactions between serotonin and other neurotransmitters such as GABA and dopamine.

Launay et al. (2008) reported that an elevated MAO in smokers than non-smokers which was demonstrated to be due to smoking induced epigenetic regulation of MAO-B i.e. a reduction of its gene promoter methylation, resulting in high MAO amounts which persist along after (over 10 years) quitting smoking. Cicin-Sain et al. (2007) reported that
both alcohol and tobacco consumption may contribute to the lowering of overall platelet MAO-B activity. The effect of alcohol is small, due to interference with substrate binding, and not alteration of catalytic activity. In contrast, the effect of cigarette smoking is pronounced and relates to the dose-dependent reduction of platelet MAO activity with no influence on its affinity. Salaspuro and Salaspuro (2004) showed the synergistic effect of alcohol drinking and smoking in vivo acetaldehyde concentration in saliva. They concluded that the co-use of alcohol and cigarette markedly increased exposure of upper digestive tract mucosa to carcinogenic salivary acetaldehyde of smoking and drinking subjects may explain the synergistic and multiplicative risk effect of alcohol drinking and tobacco smoking on upper gastrointestinal tract carcinogenesis. Experiments of Wang et al. (2004) reveal that humans of different carriers of epsilon 3 and epsilon 4 and Avail (+) alleles would have higher risk of suffering from CHD if they drink alcohol or smoke heavily. Cigremis et al. (2006) concluded that chronic exposure to ethanol and smoke may cause an oxidative burst in rat kidney by increasing the formation of reactive oxygen species. Further, Ahmed et al. (1976) reported that cardiovascular abnormalities of long-term cigarette smokers predominantly depend on nicotine of cigarettes. Alcohol and cigarette smoking, together decreases serum or plasma beta-carotene levels and vitamin E (Rimm and Colditz, 1993). Tarcan et al. (2006) results indicated a protective effect of cigarette smoking and alcohol consumption in the occurrence of clinical benign prostatic.

Decreased olfactory ability was observed in patients who use ethanol and cigarettes (Vent et al., 2003; 2004). Vanisree and Sudha (2006) reported that curcumin combats the cigarette smoke and ethanol induced lipid alterations in lung and liver in rats. Combined alcohol and tobacco consumption showed a synergistic effect and increases the cancer risk more in multiplicative than in an additive manner (Maier et al., 1990). Cooper and Magwere (2008) concluded that nicotine and alcohol consumption induced pathogenesis is mediated through nitric oxide. Several experiments reveal that chronic ethanol ingestion increases endothelial nitric oxide expression and nitric oxide production (Zima et al., 2001; Ockonomaki et al., 2004; Kleinhenz et al., 2008). Cigarette smoke induced oxidative damage is well documented (Van der Vaart et al., 2004). Several reports reveal a decrease in blood serotonin levels and also platelet MAO activity. Differential effects of nicotine on alcohol consumption in men and women were reported
Additive cardiovascular effects of ethanol and nicotine contributed to arrhythmias and sudden death in patients with coronary heart disease (Benowitz et al., 1986b). Ashakumary and Vijayammal (1996) reported that cigarette smoking and alcohol consumption had additive effect on lipid peroxidation, antioxidant defence mechanism in rats. Alcohol consumption induced changes in platelet properties and functions (Numminen et al., 1996). Nicotine was reported to decrease blood alcohol concentrations in rats (Chen et al., 2001). Wu et al. (2001) reported that cigarette smoking and alcohol consumption were confirmed to have similar effects on lipid and lipoprotein levels in Caucasians. Furthermore, joint exposure to smoking and drinking would help in predicting lipid and lipoprotein levels (Vanisree and Sudha, 2006). Walter et al. (2007) reported that the effect of alcohol and tobacco is cumulative, with higher levels of alcohol and tobacco consumption being associated with higher levels of testosterone before and after alcohol withdrawal. Whitehead et al. (1996) opined that smoking even small amounts could negate protective benefits gained from moderate alcohol consumption. Inflammatory alterations due to combined use of cigarette smoke and alcohol consumption were detectable on exfoliative cytology of the buccal mucosa in a young group demonstrating the usefulness of cytology for early detection in smokers (Pavanello et al., 2006).

Despite the fact that vast literature is available separately on alcoholism as well on cigarette smoking, limited literature is available on the effects of combined use of these substances. The precise mechanisms of action of alcohol and nicotine and several events related to these are not clear. Actual events and biochemical effects that occur in alcoholics who smoke cigarettes were not studied systematically so far. Very limited literature explicitly explored the biochemical events and related mechanisms of exposure of cigarette smoking and alcohol consumption. A potential aspect in this regard is that several other unmeasured factors such as dietary intake and physical activity might act as confounding variable or interacts with alcohol consumption and cigarette smoking to influence biochemical events. It is possible that the study population may represent a higher socioeconomic and nutritional status than the general population. Therefore the interventions often be multifactorial and targeted at high risk individuals a special in the joint exposure to cigarette smoking and alcohol consumption. Johnson et al. (1991)
reported that cigarette smoking slows gastric empting and as a consequence delays alcohol absorption. Most of the studies on cigarette smoking associated with alcohol consumption have been carried out in western societies with little published comparable data for Asian populations, in particular for Indian population. Several studies revealed association between blood lipids especially and the habits along with the joint use of alcohol and cigarette smoking which were reported to have strongly conditioned blood lipids, body weight and than non-smokers and non-alcoholics (Wannamethee and Shaper, 1992; Rimm and Colditz, 1993; Chyou et al., 1997). Rossing et al. (2000) reported that both smoking and alcohol consumption may influence thyroid function and the nature of these relations are not well understood. Nixon et al. (2007) observed cognitive enhancing effects of acute nicotine on attentional processes and alcoholics who are regular smokers and more sensitive to the effects of nicotine on cognition. Kapaki et al. (2007) reported that smoking may act synergistically in the causation of alcohol induced oxidative damage. Schroder et al. (2002) showed that association of nutrient intake, blood lipid variables with tobacco and alcohol consumption and reported that several health benefits of the diet appeared to be strongly counteracted by smoking. The combined ingestion of ethanol and cigarette smoke resulted in significant formation of smoke related DNA adducts in the esophagus and in their further dramatic increase in the heart (Izzotti et al., 1998). Moderate alcohol consumption among long term smokers may potentially decrease the risk of adenoma compared to abstainers (Austin et al., 2008). Human in vivo protein magnetic resonance spectroscopic imaging finding indicated that chronic cigarette smoking exacerbates chronic alcohol-induced neuronal viability and cell membranes in the midbrain and on cell membranes of the cerebellar vermis (Durazzo et al., 2006). Higher smoking levels are associated with metabolic concentrations in select subcortical structures.

Nitric oxide

The metabolic status of nitric oxide and functional status between oxidation and antioxidation systems in human body are in close relationship with the health (Zhou et al., 2000a). If the metabolism of nitric oxide is abnormal the dynamic balance between oxidation and antioxidation is disturbed with an increase in the concentration of free
radicals thereby causing damage or toxicity. Antioxidant machinery plays an important role in scavenging free radicals thereby protecting biological membranes against oxidation, peroxidation and lipid peroxidation (Zhou et al., 1994; 2000b). Enhanced oxidative stress, decreased antioxidant status and disturbed NO metabolism may play a major role in the events associated with alcoholism and smoking.

The role of nitric oxide in maintenance of normal physiological and pathological processes is well established. Available literature strongly suggested the involvement of NO in alcohol induced events and also in alcohol plus smoking induced events. It is well known that NO has two faces i.e. a cold gentle beneficiary one and the other a detrimental face. Nitric oxide is an omnipresent intercellular messenger in all vertebrates modulating blood flow, thrombosis and neural activity (Pacher et al., 2007). Nitric oxide (NO) is a simple, free radical gas with important bioregulatory functions in the nervous, immune and cardiovascular systems. NO synthase (NOS, EC.1.14.13.39) is an enzyme, which generates NO from the terminal guanidine nitrogen of L-arginine during its conversion to L-citrulline. Three isoenzymes of NOS (nNOS, eNOS and iNOS) have been identified. They all require NADPH, tetrahydro biopterin, flavin adenine dinucleotide and flavin mononucleotide as cofactors and all contain heme (Fostermann et al., 1994). Neuronal NOS (nNOS, type I) has an important function in neurotransmission (modulation of N-methyl-D-aspartate, NMDA receptor). NO can originate from at least four different sources in the central nervous system: the endothelium of cerebral vessels, the immuno stimulated microglia and astrocytes, non-adrenergic noncholinergic nerves and the glutamate neurons. However nitric oxide has been shown to be involved in many regulatory functions of different tissues ranging from cardiovascular system to modulation of neuronal function (Moncada, 1991; Culotta and Koshland, 1992; Wink and Mitchell, 1998).
Nitric oxide (NO') production may play a dual role mediating protective effects at lower concentrations and tissue damage by over production (Tritto and Ambrosio, 2004). 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. 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.

**Figure 4:** The multifaceted biological effects of NO
Nitric oxide (NO) is formed by different cell types in response to a variety of physiological and patho-physiological stimuli. The intake of nicotine and/or alcohol has patho-physiological effects on organ function, and the progression of alcohol-/tobacco-related diseases seem to be directly influenced by NO-mediated mechanisms. Nicotine has an adverse influence on blood vessel functionality, repair and maintenance. Chronic nicotine exposure augments atherosclerosis by enhancing the production of proinflammatory cytokines by macrophages which then activate Atherogenic NF-kB target genes in aortic lesions (Lau et al., 2006). Alcohol produces NO which speeds up the apoptosis of neutrophils. Alcohol sensitizes the liver to endotoxemic shock. Nitrosative stress and increased basal levels of NO contribute to tumour growth. The progression of disease seems to be directed via a definite NO-mediated mechanism (Cooper and Magwere, 2008).

Alcohol-induced oxidative stress and free radical generation

No single process or underlying mechanism can account for all the effects of alcohol on an organism or even on one specific organ. Instead many mechanisms act in concert reflecting the spectrum of organisms response to a myriad of direct and indirect actions of alcohol which are key factors that have been suggested to play a central role in many pathways of alcohol damage due to excessive generation of molecules called free radicals. Reactive oxygen species (ROS) are small, highly reactive, oxygen-containing molecules that can react with and damage complex cellular molecules, particularly in the liver (Wu and Cederbaum, 2003). Ethanol metabolism is directly involved in not only the production of reactive oxygen species, but also related in the formation of an environment favourable to oxidative stress such as hypoxia, endotoxaemia and cytokine release (Sergent et al., 2001). The actions of a class of oxygen species can damage or cause complete degradation (peroxidation) of essential complex molecules in the cells
including lipids, proteins and DNA. Alcohol promotes the generation of ROS and/or in several tissues such as brain, liver and stomach interfering with the body's normal defense mechanisms. Cells have several protective mechanisms to prevent ROS formation or to detoxify the ROS or by scavenging them or promoting their decomposition (Young and Woodside, 2001). These mechanisms employ molecules called antioxidants.

When a person consumes oxygen, a small percentage of oxygen is converted to ROS. The mitochondrial respiratory chain in all cells generates most of the ROS produced in the body. Super oxide anion and hydrogen peroxide produced during aerobic respiration are precursors of the hydroxyl radical with the participation of transition metals. Other sources of ROS are in liver a group of enzymes called cytP-450 mixed function oxidizer. Further, ROS also are produced by a variety of oxidative enzymes such as xanthine oxidase (Sergent et al., 2001). Other sources of ROS in the body are two types of immune cells viz., macrophages and neutrophils. Moreover, many redox cycling agents such as pesticides, medication and certain metals contribute to the generation of free radicals. Important free radicals generated in the body are the hydroxy radical (OH*), superoxide radical (O2*), nitric oxide (NO) and peroxynitryl radical (ONOO*). Cellular proteins and amino acids are sensitive to attack and oxidation by the hydroxy radical. Proteins are made up of 20 different amino acids which differ in their sensitivity to interactions with ROS for e.g. cysteine, methionine and histidine are more sensitive and the enzymes in which these amino acids are located at positions that are critical to the enzyme activity will become inactivated by the interactions with ROS. ROS are a major source of DNA damage, causing strand breaks, removal of nucleotides and a variety of modifications of the organic bases of the nucleotides. ROS lead to permanent changes or damage to the DNA with potentially detrimental effects for the cell (Wu and Cederbaum, 2003; Albano, 2006).

Alternatively loss of its three dimensional structure as well as fragmentation, aggregation or cross-linking of the proteins thereby making the proteins more susceptible for degradation or elimination of the damaged protein of the cell. Similarly lipids,
particularly polyunsaturated fatty acids present in the membrane phospholipids, are particularly sensitive to hydroxyl radicals and other oxidants (Lindi et al., 1998). A single hydroxy radical can result in the peroxidation of many polyunsaturated fatty acid molecules because the reactions involved in this process are part of a cyclic chain reaction. Lipid peroxidation is a chain reaction providing a continuous supply of free radicals that initiate further peroxidation and the whole process involves three steps, initiation, propagation and termination. Whether lipid peroxidation leads to generation of free radicals or vice-versa is still not clear. Whatever may be the cause this ultimately leads to tissue damage by causing deleterious effects (Reinke, 2002; Wu and Cederbaum, 2003).

The metabolism of alcohol is inherently associated with the production of both reactive oxygen and nitrogen species (ROS and RNS) resulting in oxidative and nitrosative stress (Das and Vasudevan, 2007). Nitric oxide plays a mediating role in alcohol-related diseases and tissue damage including cancer (Pöschl and Seitz, 2004; Yuan et al., 2006) and oxidative liver injury (Dey and Cederbaum, 2006). The work of Polikandriotis and colleagues highlights mechanisms through which chronic alcohol consumption might be involved in incidences of acute respiratory distress syndrome and lung injury. They first showed that chronic ethanol stimulated NO production via PI-3 kinase and hsp90-dependent induction of eNOS in porcine pulmonary artery endothelial cells (Polikandriotis et al., 2005). Using rat models they further showed that alcohol increased hydrogen peroxide production, eNOS expression and activity, as well as markers of oxidative stress and damage in lungs of male Sprague-Dawley rats (Polikandriotis et al., 2006; 2007). In Caco-2 intestinal cells in vitro, ethanol was shown to induce iNOS expression as well as NO synthesis resulting in oxidative/nitrosative stress and disruption of microtubules formation and intestinal barrier function (Banan et al., 2000). The relationship between chronic ethanol, oxidative stress, and tissue injury has thus been established. Some results have however shown negative effects of ethanol on eNOS expression; chronic alcohol downregulated eNOS protein levels and combined synergistically with LPS to lower eNOS activity (Karaa et al., 2005) and transcription (Zhao et al., 1997) in male Sprague-Dawley rats suggesting that alcohol consumption might sensitize the liver to endotoxemic shock.


Toth, K.M., Berger, E.M., Beehler, C.J & Repine, J.E (1986): Erythrocytes from cigarette smokers contain more glutathione and catalase and protect endothelial cells from


