DISCUSSION
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Cigarette smoking is a recognized risk factor for cardiovascular diseases, lung cancer, pulmonary diseases and osteoporosis leading to millions of death worldwide.\textsuperscript{9} Reports revealed that 80% of cigarette smokers are confirmed alcoholics and cigarette smoking has been consistently associated with alcohol abuse.\textsuperscript{8} Therefore combined use of alcohol and cigarette smoking is common and world-wide. The precise events and mechanisms related to the interactions of cigarette smoke and alcohol in humans who use both cigarettes and alcohol are yet to be understood fully.\textsuperscript{1} Hence in present study an attempt has been made to evaluate the impact of cigarette smoking on alcoholics, in particular the biochemical events and alcohol-cigarette smoke interactions in chronic users of alcohol and cigarettes.

Glucose homeostasis Vs combined exposure to alcohol and cigarettes:

Results of the study clearly demonstrated that smokers with alcohol intake showed higher fasting plasma glucose (140 mg/dl Vs 130 mg/dl) and HbA1C (7.2 mg/dl Vs 5.2 mg/dl) levels than smokers without alcohol suggesting the impact of cigarette smoking in alcoholics. It is also pertinent to note that alcoholics, smokers and alcoholic smokers had increased plasma glucose concentrations when compared to controls.

It is well known that maintenance of stable levels of blood glucose is a complex and a finely regulated one among all homeostatic mechanisms in which various hormones, enzymes, factors and tissues play a role. Hike in blood glucose concentration in alcoholic smokers and the other two experimental group’s viz., alcoholics and smokers when compared to controls strongly suggested a disturbance in glucose homeostasis as well as derangement in carbohydrate metabolism in all the experimental groups’ viz., smokers, alcoholics and alcoholic smokers. In fact, increased in plasma glucose may be chiefly due to decreased
utilization of glucose by peripheral tissues and/or increased glycogenolysis and/or due to an increase in absorption of dietary glucose. Though alcohol is a hypoglycemic drug several reports revealed that excessive and chronic alcohol consumption results in hyperglycemia. Earlier studies suggested that both the cigarette smoking and alcohol use separately lead to zigzags in plasma glucose concentrations with disturbances in carbohydrate metabolism, in particular glucose metabolism. This study for the first time reported increase in disturbance in plasma glucose in alcoholics by cigarette smoking. Similarly higher HbA1C levels in alcoholic smokers not only suggested disturbance in glucose homeostasis, but also indicated glycation of proteins affecting the function of several proteins. The glycosylation of haemoglobin occurs by a non-enzymatic reaction between glucose and amino terminal valine of β-chain. This on rearrangement resulting protein called HbA1C presents the picture of blood glucose homeostasis. Collagen fibers, antithrombin-III are also glycosylated. These changes may possibly favor the accelerated blood vessel damage that occurs in alcoholics as well as cigarette smokers. Observed increase in glucose and HbA1C in alcoholic smokers and smokers in comparison with controls in the present study strongly suggested that alcoholic smokers are prone/ susceptible to diabetes mellitus. Vast literature on cigarette smoking suggested the damage of all tissues and organs by cigarette smoking or alcohol use due to induction of free radicals. A puff of cigarette smoke introduced $10^{18}$ free radicals, with chief constituents of nicotine, NOx, aldehydes, peroxides, benzene, and epoxides, into the human body.\textsuperscript{11,12,32} It is well known that alcohol induced oxidative stress due to free radicals followed by nitrosative stress along with depleted antioxidants in the body worsens the situation leading to the damage of all tissues in particular the liver along with pancreas and insulin production.

Studies of Bornamisza and Suciul suggested that cigarette smoking induced release of catecholamines, STH and cortisol were responsible for the
observed hyperglycemia and the effect was reported to be more pronounced among diabetics. Besides the operation of some protective mechanisms regulating glucose metabolism were reported by some workers. 3 Persson et al., have reported that heavy users of cigarettes or moist snuff have an increased risk of type II diabetes with impaired glucose tolerance. 23 Though cigarette smoke does contain nitric oxide as a component it is pertinent to note from the earlier studies of that smoking induces dysfunction of nitric oxide biosynthesis and upregulation of endothelial nitric oxide synthase contributed to the elevated nitric oxide production. Acute bouts of smoking have been shown to provoke hyperglycemia. Rincon et al have reported a profound effect of cigarette smoking on glucose transport in skeletal muscle and basal glucose transport is markedly elevated, and insulin stimulated glucose transport is impaired in habitual cigarette smokers. 27

Lipids and lipoprotein patterns Vs combined use of cigarettes and alcohol:

Higher concentrations of triglycerides and cholesterol were recorded in alcoholic smokers than other groups namely viz., alcoholics and smokers suggesting risk of cardiovascular problems. Observed increase in LDL-C, VLDL-C with a decrease in HDL-C in alcoholic smokers indicated cardiovascular risk in this group than smokers and alcoholics who were also undoubtedly at the risk. These findings are in agreement with earlier studies of Suzane et al., 2007, Porkka et al., 1996 and others who reported increased TC, TG, VLDL-C,LDL-C levels and decrease in HDL-C and apolipoprotein A-1 levels. Thus combined or couse of alcohol and cigarettes exert adverse effects on LDL-C and HDL-C metabolism and generally it is expected that the effects of one could be modified by the influence of the other. 24,31 Observed significant hike in plasma cholesterol in the present study indicates the interference of cigarette smoking and alcohol use in cholesterol synthesis and/or utilization and this finding is in agreement with earlier reports. 6,7,19 Increased
cholesterogenesis due to increased activity of HMG CoA reductase might have led to the present observation. In addition, decrease in hepatic degradation of cholesterol to bile acids due to nicotine administration was observed by Latha et al. (1999). Der-min Wu et al., (2001) reported increase in triglycerides accompanied by decrease in LDL-C levels in plasma in drinkers with heavy smoking habit. Alcohol consumption alone exerts an independent influence on the HDL-C level. Our results strongly suggested the significance of joint exposure to cigarette smoking and alcohol consumption in predicting lipid and lipoprotein levels.

Circulatory levels of VLDL, LDL and HDL-cholesterols are considered to be powerful risk factors for cardiovascular diseases (CVD). Observed increase in total cholesterol, LDL-C, VLDL-C, triglycerides with significantly decreased HDL-C in alcoholics of the present study suggested cardiovascular risk in these groups. Furthermore, results of this study showed increase in the levels of plasma cholesterol, triglycerides with a decrease in phospholipids. In general, hyperlipidemia is a complication of alcohol toxicity leading to cardiovascular problems and other abnormalities. Accumulation of fat in the liver in chronic alcoholics acts as stimulus for the secretion of lipoprotein into the blood stream and also the development of hyperlipidemia. In general, HDL is considered to be a beneficial protein helps in scavenging cholesterol from extra hepatic tissues in presence of Lecithin Cholesterol Acyl Transfarase (LCAT) and brings it to liver. Increased plasma cholesterol, triglycerides, VLDL, LDL and atherogenic index with a decrease in HDL concentration observed in alcoholics in comparison with teetotalers suggested cardiac risks as well as hepatic dysfunction in alcoholics. Also in the present study, alcoholics showed increased NO production (elevated levels of nitrite and nitrate) when compared to controls. NO mediated regulation in hepatic production or secretion of apolipoprotein particles, increase in triglyceride lipases
and also removal of circulating HDL might have played a role in the observed effect.10,20

Increased activities of ALP, SGOT and SGPT of plasma of alcoholic smokers strongly suggested tissue damage to the liver. All these enzymes are well known markers. The massive release of ROS and NO of cigarette smoke and alcohol may be responsible for the observe affect. The increased daily smoking amounts increases SGOT and SGPT.13

**Oxidative stress Vs combined use of alcohol and Cigarettes:**

Increased lipid peroxidation in plasma and erythrocyte membrane of smokers observed in the present study than other two groups' viz., alcoholic smokers and alcoholics suggested the potential risk due to oxidative stress by cigarette smoke. Higher lipid peroxidation in plasma as well as red cell membrane in smokers suggested the extent of free radical activity and susceptibility of lipids to peroxidation reflect increased oxidative damage in smokers consuming alcohol as well as in other groups' viz., smokers and alcoholics. Changes in lipid profile increased LDL, decreased HDL followed by increased lipid peroxidation support the hypothesis that the atherogenic effects of smoking are mediated in part by free radical damage to lipids.30 Besides depleted antioxidants and other factors may aggravate the process.17, 21, 25 Presumably as a result of these oxidative events increased formation of oxidized proteins, lipids and DNA i.e., oxidative damage has been observed in smokers. In principle antioxidant supplementation should be able to prevent some of this oxidative damage.2,5,14,26 In general, smokers were reported to possess lower levels of vitamin E and supplementation of vitamin C and vitamin E and β-carotene decrease the extent of lipid peroxidation in smokers to base line levels of non smokers after only a few weeks of supplementation.11,29

**Nitrosative stress in alcoholic smokers:**

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Increased nitrite and nitrate concentrations in plasma and red cell lysate reflecting increased production of NO (nitric oxide) is another important observation in the present study in alcoholic smokers. Observed higher NO production in smokers and alcoholics in present study were also in agreement with earlier reports (re). NO is a free radical gas and is constitutively produced from endothelial cells. NO can diffuse to long distances with loss in its mass. Now the multifaced actions of NO are well established. Besides the effectiveness of nitric oxide in preventing peroxidation of lipid by serving as potent scavenger of alkoxyl (LO, Peroxyl LOO) radicals was reported. In addition NO scavenges some other radicals such as tyrosine, tryptophan Hence the magic role of NO is complex to understand under physiological conditions.12

Though nicotine is considered to be the chief constituent responsible for cigarette smoking addiction, available literature strongly suggest relationship between nitric oxide and nicotine cigarette smoking addiction, and also forward a hypothesis that NO contributes to smoking addiction. According to this hypothesis smokers are first exposed to high concentrations of inhaled NO from smoke and second to endogenously release NO after uptake of nicotine in to the brain. As a result endogenous NO synthesis in airways and blood vessels of smokers is reduced. Subsequently, because NO is involved in maintaining airway dilation smokers may have constricted airways.4 During smoking, however, NO from smoke may dilate the constricted airways allowing the smoke an easier passage into the lungs and exposing the body and the brain to more nicotine. NO can endogenously be released by nicotine in nervous tissue, and may decrease sympathetic output of brain which is associated with stress reduction. This second form of exposure to NO also inhibits the reuptake of dopamine which may contribute to dopaminergic receptor stimulation and thus to acute rewarding effects of nicotine. Role of nitric oxide in nicotine addiction was also supported by the finding that in animals NO synthase (NO inhibitors) attenuate symptoms of
nicotine abstinence syndrome. Hence it has been suggested that low arginine diet might be useful for the treatment of nicotine addiction.32

In various earlier studies a therapeutic role of nicotine was reported. Nicotine is the chief addictive component of cigarette smoke but one among several thousand components and in fact nicotine effects are not equivalent to whole cigarette smoke. In case of nicotine, inhaled nicotine may maintain circulating nitric oxide in humans. Nicotine could alter the production of nitric oxide via nicotine receptor activation of nitroxidergic nerves of bypass receptor activation by directly interacting with biochemical pathways in endothelial cells and/or through production of oxygen derived free radicals and thereby nicotine could affect production of nitric oxide by interaction with NOS. Literature also reveals that β2 containing neuronal acetylcholine receptor is involved in mediating reinforcing capacity of nicotine by the release of the neurotransmitter dopamine.22 Combined exposure to alcohol and smoke enhanced oxidative stress, adversely affecting the antioxidant defense system and thus is likely to be more harmful than the effect of alcohol or smoke alone.

**Erythrocyte membrane protein moiety and carbonyl contents in alcoholic smokers:**

Though R.B.C membrane protein concentrations of three experimental groups viz., alcoholic smokers, smokers and alcoholics was reported to be decreased when compared to controls, the decrease in protein moiety of alcoholic smokers was found to be much less in comparison with that of smokers and alcoholic groups. This finding clearly demonstrated the impact of smoking in alcoholics. The minergestic effect of alcohol and smoking is obvious from the results of this study. However, the precise mechanism of the decrease in membrane protein moiety requires further in depth study. Membrane carbonyl content of alcoholic smokers was found to be increased significantly in alcoholic smokers (1.54nmol/mg
protein), alcoholics (1.21 nmol/mg protein) and smokers (1.3 nmol/mg protein) when compared with controls indicating the exposure of carbonyl groups for interaction with other biomolecules facilitating various biochemical events in experimental groups, in particular alcoholic smokers. Strongly suggesting the intra membrane interactions of cigarette smoking and alcohol.

Effect of phytoextracts of *Phyllanthus emblica* and *Pterocarpus santalinus* on oxidative damage in smokers, alcoholic smokers and alcoholics:

Based on literature and earlier studies in our laboratory the phytoextracts of *Pterocarpus santalinus* and *Phyllanthus emblica* were selected to investigate their possible therapeutic affects against the damage induced by alcohol, cigarette smoking and the combined use of alcohol and cigarettes in humans. Phytochemical analysis of the extracts of *Pterocarpus santalinus* and *Phyllanthus emblica* revealed the presence of beneficial phytocompounds terpenoids, tannins, phenols, saponins, flavanoids, steroids, alkaloids and photobalanins with good therapeutic potential. The concentrations of alkaloid, flavanoid, terpenoid and saponins in *Pterocarpus santalinus* and *Phyllanthus emblica* were reported to be 10 ug, 35 mg, 6 mg, 0.44mg/gm and 2.5ug, 42.3 mg, 5.4 mg, 1.32 mg/gm respectively. All these components appear to be responsible for the observed therapeutic effects and these phytocompounds ameliorate the damage caused by alcohol as well as cigarette smoking. The precise events and mechanism(s) by which therapeutic effects are exerted or yet to be understood fully.

Further more, results of the present study clearly demonstrated the protective affect of aqueous extracts of *Pterocarpus santalinus* heart wood powder and fruit of *Phyllanthus emblica* against ethanol induced haemolysis of human erythrocytes. When normal human erythrocytes pre incubated in aqueous extract of *Pterocarpus santalinus* and *Phyllanthus emblica* (125ug/ml) different
concentrations of sodium chloride ranging from 0.1% to 0.9% and extent of
haemolysis was examined to assess the influence of *Pterocarpus santalinus* and
*Phyllanthus emblica* on membrane tolerance/osmotic fragility of red blood cells.
Results of the study (Figs- 4, 5, 6) revealed a decrease in tendency of hemolysis at
various concentrations of sodium chloride solution suggesting the protective effect/
tolerance conferred to red blood cell membrane. Probably and possibly several
specific and nonspecific active principles present in these extracts are responsible
for the therapeutic effects of these phytoextracts and a detailed indepth study is
required to understand the precise events and mechanism(s) of therapeutic actions
of these phytoextracts.

In all this study strongly suggests that cigarette smoking and
alcohol use adversely affect human health leading to cardiovascular problems and
damage of all tissues. Moreover this study strongly demonstrates cigarette smoke
induced exacerbation of the damage in alcoholics. Furthermore use of phytoextracts
in particular aqueous extracts of *Pterocarpus santalinus* and *Phyllanthus emblica* to
counteract the adverse effects of alcohol and cigarettes, and also for alleviation
of the damage caused by the use of cigarettes and alcohol.

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