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# Modulation of Lecithin Activity by Vitamin-B Complex to Treat Chronic Ethanol Induced Oxidative Stress in Lung

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**Abstract: Background:** Alcohol abuse is a systemic disorder and increases the risk of lung injury. Derangement in glutathione homeostasis is a potential link between chronic alcohol ingestion associated oxidative stress and increased susceptibility to acute lung injury. One of the contributing factors of glutathione depletion is decreased methionine metabolism. In this study, we investigated the efficacy of lecithin, an important class of phospholipids containing choline and a source of methionine, with Vitamin-B complex for the treatment of long term deleterious effects of ethanol in the lung. **Results:** Ethanol (1.6 g ethanol/kg body wt/day for 24 weeks) significantly affects body weight of 16-18 week old male albino rats of Wistar strain weighing 200-220 g. The levels of TBARS, nitrite, protein carbonyl, oxidized glutathione (GSSG), redox ratio (oxidized to reduced glutathione ratio) and Glutathione S-Transferase (GST) activity elevated; while reduced Glutathione (GSH) level and activities of Glutathione Reducatase (GR), Glutathione Peroxidase (GPx), catalase, Superoxide Dismutase (SOD) and $Na^+K^+$ATPase were reduced significantly due to ethanol exposure compared to the control group. Histopathologic examination revealed severity of inflammation in the lung. The dietary supplementation with lecithin, a glutathione precursor decreased ethanol-induced oxidative stress, prevented inflammatory response and thereby limited lung injury. **Conclusion:** However, supplementation of trace amount of vitamin-B complex with lecithin showed more protective effect in reversing the chronic ethanol-induced oxidative stress mediated effects. Together, these findings persuasively argue that lecithin with vitamin-B complex is a promising therapeutic approach in controlling ethanol-induced oxidative stress in the lung.

**Key words:** Ethanol, glutathione, lecithin, lung, oxidative stress, Vitamin-B complex

## INTRODUCTION

The deleterious health effects of alcohol consumption result in irreversible organ damage (Guidot and Hart, 2005). By contrast, the ravages of alcohol abuse have been viewed as relatively sparing the lung. It had been generally assumed that chronic alcohol abuse had no effect on the lung itself (Joshi and Guidot, 2007). But two epidemiological studies revealed that alcohol abuse independently increased the risk for developing the Acute Respiratory Distress Syndrome (ARDS) (Moss *et al*., 1996, 2003), a devastating form of acute lung injury in which the air spaces become flooded with inflammatory cells and debris, alveolar epithelial cell dysfunction, including disruption of the alveolar epithelial barrier, leading to respiratory failure and may cause death (Ashbaugh *et al*., 1967). Alcohol abuse is now being considered as a systemic disorder and increases the risk of lung injury (Das, 2009).

Chronic alcohol ingestion is associated with oxidative stress in the lung (Das, 2009). Derangement in glutathione homeostasis is a potential link between chronic alcohol abuse and an increased susceptibility to acute lung injury. Decreased glutathione availability in the alcoholic lung contributes to not only decreased function but decreased viability via oxidative stress (Brown *et al*., 2007). Glutathione replacement would seem to be an obvious choice in view of the extensive evidence implicating glutathione depletion in the pathophysiology of alcohol-induced liver and lung dysfunction (Guidot and Brown, 2000; Guidot *et al*., 2000; Holguin *et al*., 1998). One of the contributing factors of glutathione depletion is decreased methionine metabolism (Tsukamoto and Lu, 2001). Though the methionine metabolic cycle is present in all tissues (Finkelstein, 2000), the liver plays a central role in methionine metabolism, as half of the daily methionine is catabolized here (Tsukamoto and Lu, 2001). Furthermore, the lung is

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dependent on hepatic synthesis of glutathione because it imports glutathione from the plasma (Holguin *et al*., 1998). The first step in methionine metabolism is the formation of S-adenosylmethionine (SAM) catalyzed by methionine adenosyltransferase (MAT). SAM is converted to homocysteine (Finkelstein, 1990; Mato *et al*., 1994).

There are three pathways that metabolize homocysteine. One is the trans-sulfuration pathway, which converts homocysteine to cysteine only in the liver and lens (Lu, 1999). Homocysteine condenses with serine to form cystathionine by cystathionine β-synthase (CBS) (Lu, 1999; Mato *et al*., 1997). Cleavage of cystathionine by γ-cystathionase then release free cysteine, the rate-limiting precursor for GSH synthesis (Lu, 1999). The other two pathways resynthesize methionine from homocysteine (Mato *et al*., 1994, 1997).

An alternate salvage pathway for homocysteine methylation occurs in the liver and kidney, where choline is precursor to betaine, the substrate for betaine homocysteine methyltransferase (BHMT). Endogenous betaine is maintained as a product of choline, which in turn is provided in the diet or as an endogenous product of Phosphatidyl Choline (PC), the major phospholipids constituent of cell membranes. Methionine that enters from the diet or is synthesized by the Methionine Synthase (MS) and BHMT reactions is converted to SAM by MAT (Lu, 1999).

Malnutrition is a constant accompaniment of alcoholism, it is logical to question whether the prognosis of alcohol induced organ damage can be improved by provision of nutritional support along with the source of antioxidants. Prevention of oxidative stress along with correction of nutritional deficiency is one of the proposed mechanisms of therapeutic approach. We have already shown that lecithin with Vitamin-B complex was better treatment option for ethanol induced oxidative stress in the liver compared to lecithin treatment alone (Das and Vasudevan, 2006a).

In this study, we have investigated the efficacy of lecithin with vitamin-B complex for the treatment of long term deleterious effects of ethanol in the lung.

## MATERIALS AND METHODS

**Chemicals:** Ethanol was purchased from Bengal Chemicals, Kolkata. Chemicals from Sisco Research Laboratory (SRL), India, Sigma Chemical Co., St. Louis, USA; and E. Merck were used.

**Animals and treatment:** The male albino rats (16-18 weeks old) of Wistar strain weighing 200-220 g were housed in plastic cages inside a well-ventilated room, with the room temperature maintained at 25±2°C, 60-70% RH, with a 12 h light/dark cycle. Animals had free access of standard diet (Das and Vasudevan, 2006a, b) containing 31% Bengal gram, 30% gingelly oil cake, 28% wheat, 10% polished rice, 0.5% salt mixture, 0.3% vitamin-mineral mixture, and 0.2% yeast with fish or liver oil. Food and water were given *ad libitum*. Animals were weighed daily and their general condition was recorded including their daily intake of liquid.

The rats were divided into the following four groups of 6 animals each. Group I served as control and were fed normal diet, water and isocaloric glucose solution instead of ethanol (1.6 g $kg^{-1}$ body wt); Group II mice were treated with ethanol (1.6 g ethanol/kg body wt/day for 24 weeks); Group III mice were treated with lecithin+ethanol (1.6 g ethanol and 500 mg lecithin together/kg body wt/day for 24 weeks); Group IV mice were treated with lecithin+Vitamin B-complex+ethanol (1.6 g ethanol and 500 mg lecithin mixed with Vitamin-B complex together/kg body wt/day for 24 weeks).

Vitamin-B complex consisting of thiamine mononitrate (10 mg), riboflavine (10 mg), pyridoxine hydrochloride (3 mg), cyanocobalamin triturate (15 mcg), nicotinamide (45 mg) and calcium pantothenate (50 mg) was supplemented per kg body weight. Ethanol, lecithin or Vitamin-B complex of desired concentrations were freshly dissolved in distilled water and fed orally by intragastric infusion technique.

### Methods

**Experimental procedure:** The rats were sacrificed after over-night fast at the end of experimental period, by applying intraperitoneal Na-pentobarbital (Nembutal, 60 mg $kg^{-1}$ body weight) (euthanasia) (Fortunato *et al*., 2007). The lung was dissected out, cleaned with ice-cold saline, blotted dry, and immediately transferred to the ice chamber for preservation. Various oxidative stress related non-enzymes and enzymes were estimated. The Animal Ethics Committee of the Institution approved the procedures in accordance with the CPCSEA guideline.

**Biochemical methods:** Lung was homogenized in 0.25 M sucrose solution, diluted with 0.9% saline and tissue protein was estimated (Lowry *et al*., 1951).

**Determination of lipid peroxidation (LPO):** Lung sample was homogenized in ice-cold 0.25 *M* tris buffer (pH 7.4). 0.3 mL of this homogenate was mixed with 2 mL of TCA-TBA-HCl [trichloroacetic acid (TCA) 15% w/v, thiobarbituric acid (TBA) 0.375% w/v, and hydrochloric acid (HCl) 0.25 N], heated for 15 min in a boiling water bath, cooled; the flocculent precipitates were removed, and the absorbance was recorded at 535 nm. The extent of

lipid peroxidation was calculated using molar extinction coefficient $1.56X10^5$ $M^{-1}cm^{-1}$ (Sinnhuber *et al*., 1958).

**Nitrite estimation:** Sulfanilamide (1%, 50 μL) in 2.5% ortho-phosphoric acid (Griess reagent 1) was added to the tissue homogenate, followed by N-(1-naphthyl) ethylenediamine (0.1%, 50 μL) in distilled water (Griess reagent 2) was added, incubated in dark at room temperature for 10 min. The absorbance was measured at 540 nm. The concentration of nitrite was measured by using $NaNO_2$ as a standard (Das *et al*., 2010).

**Protein-carbonyl content:** Protein was precipitated with 20% trichloroacetic acid and centrifuged. The precipitate was resuspended in 2,4-dinitrophenylhydrazine (10 mM) and vortexed at 10 min intervals for 1 h at room temperature. The pellet was washed thrice with ethanol/ethyl acetate to remove the free reagent, resuspended in 0.6 mL of 6 M guanidine hydrochloride, incubated at 37°C for 15 min and centrifuged at 5,000 g for 3 min. The absorbance of supernatant was measured at 366 nm for carbonyl content, and calculation was performed with $\epsilon$ value of 22,000 $M^{-1}cm^{-1}$ (Bhatwadekar and Ghole, 2005).

**Glutathione content:** The lung (~100 mg) sample was homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4). For glutathione (GSH) content, the homogenate was immediately mixed with sulfosalicylic acid, shaked well, centrifuged, and the supernatant was mixed with 5,5'-dithiobis(2-nitrobenzoic acid) (in 0.01 M phosphate buffer, pH 8) and absorbance was recorded at 412 nm (Ellman, 1959). For oxidized glutathione, 200 μL supernatant was added to 3.78 mL water to which 40 μL 2-vinylpyridine was mixed to mask the GSH and left at room temperature for 3 h before estimation as described above (Teare *et al*., 1993).

**Glutathione reductase (GR, EC 1.6.4.2) activity:** The tissue was homogenized in phosphate buffer (0.12 M, pH 7.2) and were added to 15 mM EDTA in phosphate buffer, and 9.6 mM NADPH. The reaction was initiated by adding oxidized glutathione (65.3 mM GSSG). Change in absorbance was monitored at 340 nm and the specific activity was determined using extinction coefficient for NADPH of 6.22 $cm^{-1}$/μmole (Goldberg and Spooner, 1983).

**Glutathione peroxidase (GPx, EC 1.11.1.9) activity:** The activity was measured based on the principle that oxidized glutathione produced by GPx is reduced at a constant rate by glutathione reductase with NADPH as a cofactor. The NADPH allows the maintenance of predictable levels of reduced glutathione. The oxidative rate of NADPH was monitored at 340 nm (Paglia and Valentine, 1967).

**Glutathione-s-transferase (GST; EC 2.5.1.18) activity:** The tissue was homogenized using phosphate buffer (0.05 M, pH 6.5). 1-Chloro-2,4-dinitrobenzene (CDNB) in phosphate buffer was mixed with reduced glutathione, and then tissue extract was added. The change in absorbance was monitored at 340 nm, and activity was calculated from extinction coefficient 9.6 $mM^{-1}$ $cm^{-1}$ (Habig *et al*., 1974).

**Catalase (EC 1.11.1.6) activity:** The tissue was homogenized in 0.05 M phosphate buffered saline (pH 7.0). The rate of decomposition of $H_2O_2$ (2 μL, 30%) in 0.05 M phosphate buffer (1 mL, pH 7.0) by the homogenized tissue at 240 nm was noted. The specific activity was calculated assuming molar extinction coefficient 22,000 $M^{-1}cm^{-1}$ at 240 nm (Das and Vasudevan, 2006a).

**Superoxide dismutase (SOD, EC 1.15.1.1) activity:** The activity was measured by the inhibition of auto-oxidation of 0.2 mM pyrogallol (air equilibriated) in 50 mM at Tris-HCl buffer (pH 8.2) containing 1 mM diethylenetriamine pentaacetic acid. The rate of autooxidation was monitored at 420 nm. The percentage inhibition of the rate of pyrogallol was initiated by addition of tissue homogenate (Marklund and Marklund, 1974).

**$Na^+$-$K^+$ ATPase (EC 3.6.1.3) activity:** The tissue was homogenized in freshly prepared buffer containing: 0.25 M sucrose; 30 mM histidine and 2.4 mM sodium deoxycholate (pH 6.8). The homogenates were filtered in a double layer of gauze prior to incubation at 37°C for 15 min in a medium containing: 100 mM NaCl; 20 mM KCl; 6 mM $MgCl_2$; 6 mM ATP disodium salt (vanadium-free) and 10 mM imidazole (pH 7.8). The activity was calculated from the difference between the amount of inorganic phosphate released in the presence and in the absence of KCl and was expressed as micromoles of inorganic phosphate/mg protein/h (Katz and Epstein, 1967).

**Histopathological examination:** Lung tissues were fixed in 10% formalin, routinely processed and embedded in paraffin. Sections were cut (4 μm thick) and stained with hematoxylin and eosin to assess morphological changes under microscope.

**Statistical analysis:** Results were expressed as Mean±SD. All the statistical analysis were performed by one-way Analysis of Variance (ANOVA) with multiple comparison tests and Student's 't' test using the Statistical Package for Social Sciences, version 11 (SPSS, Chicago, Illinois). The values of significance were evaluated with p-values. The differences were considered significant at $p<0.05$.

In the present study, group I served as normal control, while group II served as experimental control. Level of significance mentioned in the text is compared to group II values. Detailed analysis is given in the corresponding tables.

## RESULTS

Body weight of ethanol (1.6 g ethanol/kg body wt/day for 24 wks) exposed rats decreased significantly by 29.12%, compared to the control group (Fig. 1). Simultaneous treatment of ethanol with lecithin or lecithin and Vitamin-B complex increased body weight by 21.7% and 22.48% respectively compared to ethanol exposed group (Fig. 1). However, there was no significant change in relative weight (g/100 g body weight) of lung due to ethanol exposure or different treatment options (Fig. 2).

Ethanol (1.6 g ethanol/kg body wt/day) treatment for 24 wks significantly increased the levels of Thiobarbituric Acid Reactive Substances (TBARS), nitrite, protein carbonyl, oxidized Glutathione (GSSG) and redox ratio, while decreased reduced Glutathione (GSH) content in the lung homogenate in comparison to the control group (Table 1). Simultaneous treatment of lecithin with ethanol significantly reversed the levels of nitrite,protein carbonyl, reduced Glutathione (GSH), oxidized Glutathione (GSSG) and redox ratio compared to ethanol treated rats (Table 1). However, vitamin-B complex supplementation in the lecithin containg ethanol not only further improved these parameters from the deleterious effect of ethanol, but also significantly reversed TBARS level compared to ethanol treated rats (Table 1).

Effects of ethanol and different test combinations on oxidative stress associated enzyme activities are presented in the Table 2. Ethanol (1.6 g ethanol/kg body wt/day) treatment for 24 wks significantly reduced the activities of Glutathione Reducatase (GR), Glutathione Peroxidase (GPx), catalase, superoxide dismutase (SOD) and $Na^+K^+$ATPase, while increased Glutathione S-Transferase (GST) activity in the lung homogenate in comparison to the control group (Table 2). Simultaneous treatment of lecithin with ethanol significantly reversed all these parameters; and Vitamin-B complex supplementation in the lecithin containg ethanol further improved these parameters against the deleterious effect of ethanol (Table 2).

Histopathological analysis showed that the brocholar and normal alveolar structure was preserved in the control specimen (Fig. 3a), wheras degenerative alveolar structures was observed in the lung tissues of the ethanol exposed groups (Fig. 3b). Treatment of lecithin with ethanol partially protected lung from deletrious effect of ethanol alone (Fig. 3c). However, lecithin with vitamin-B complex supplementation protected lung in better way than lecithin alone as evidenced on histological examination (Fig. 3d).

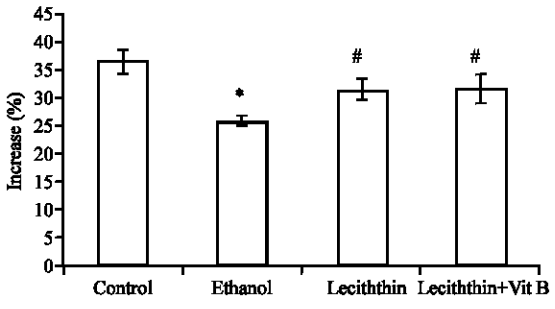


Fig. 1: Change in the body weight in different groups of animals exposed to different treatment. p-values: *<0.05 compared to control group; #<0.05 compared to ethanol treated groups

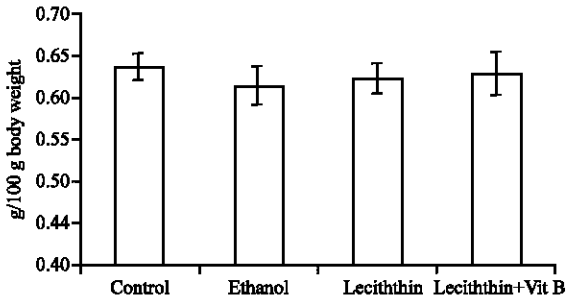


Fig. 2: Change in the lung weight in different groups animals exposed to different treatment. No significant was aboserved in either group

Table 1: Effect of lecithin and lecithin with vitamin-B complex on thiobarbituric acid reactive substances (TBARS), nitrite, protein-carbonyl, reduced glutathione (GSH) and oxidized glutathione (GSSG) levels and on redox ratios in lung homogenate

| | TBARS* | Nitrite# | Protein-Carbonyl/ | GSH$^{\Psi}$ | GSSG$^{\Psi}$ | Redoxratio(GSSG/GSH) |
|---|---|---|---|---|---|---|
| Control | 0.51±0.01 | 13.46±0.54 | 0.95±0.11 | 6.95±0.69 | 0.57±0.011 | 0.08±0.01 |
| Ethanol | 0.95±0.12$^{a}$ | 29.08±1.67$^{a}$ | 2.2±0.14$^{a}$ | 3.88±0.26$^{a}$ | 0.69±0.014$^{a}$ | 0.18±0.01$^{a}$ |
| Lecithin | 0.83±0.1$^{a}$ (-12.63) | 20.45±1.12$^{ac}$ (-29.67) | 1.56±0.11$^{ac}$ (-29.1) | 5.02±0.28$^{ad}$ (+29.38) | 0.63±0.014$^{ac}$ (-8.69) | 0.12±0.01$^{ac}$ (-33.33) |
| Lecithin + vit B | 0.71±0.05$^{bd}$ (25.26) | 16.95±0.72$^{ace}$ (41.71) | 1.45±0.12$^{ac}$ (-34.1) | 5.52±0.35$^{ac}$ (+42.26) | 0.59±0.015$^{cf}$ (-14.49) | 0.11±0.01$^{bc}$ (-38.88) |
| F variance | 29.228 | 220.527 | 105.272 | 51.32 | 81.556 | 72.149 |
| Significance | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Values are Mean±SD of 6 rats in each group. Values in the parentheses are % increase (+) or decrease (-) compared to ethanol treated group. p-values: $^{a}$< 0.001, $^{b}$< 0.01 compared to control group; $^{c}$< 0.001, $^{d}$< 0.01 compared to 24 week ethanol treated group; $^{e}$< 0.001, $^{f}$< 0.01 compared to lecithin and ethanol treated group for 24 weeks. *µmole MDA formed/min/100 mg tissue; #nmole/g wet tissue; /nmole/mg protein; $^{\Psi}$µg/mg tissue

Table 2: Effect of lecithin and lecithin with vitamin-B complex on glutathione reductase (GR), glutathione peroxidase (GPx), glutathione s-transferase (GST), catalase, superoxide dismutase (SOD) and $Na^+K^+$-ATPase activities in lung homogenate

| | GR* | GPx* | GST# | Catalase$^f$ | SOD$^{\Psi}$ | $Na^+K^+$-ATPase$^{\omega}$ |
|---|---|---|---|---|---|---|
| Control | 14.33±1.16 | 26±1.6 | 4.35±0.1 | 3.35±0.1 | 1.34±0.04 | 223.17±7.57 |
| Ethanol | 10.05±0.8$^a$ | 13±0.75$^a$ | 7.11±0.2$^a$ | 2.65±0.1$^a$ | 0.89±0.07$^a$ | 124.67±6.02$^a$ |
| Lecithin | 13.13±0.76$^c$ (+30.64) | 19.2±0.78$^{ac}$ (+47.69) | 5.75±0.1$^{ac}$ (-23.65) | 2.91±0.11$^{ad}$ (+9.81) | 1.17±0.07$^{bc}$ (+31.46) | 172.5±7.31$^{ac}$ (+38.36) |
| Lecithin + vit B | 13.92±0.94$^c$ (+38.5) | 20.35±1.1$^{ac}$ (+56.53) | 5.35±0.1$^{ace}$ (-24.75) | 3.06±0.13$^{bc}$ (+15.47) | 1.24±0.06$^c$ (+39.32) | 193.67±9.24$^{acf}$ (+55.34) |
| Fvariance | 26.106 | 137.484 | 422.232 | 37.74 | 56.418 | 177.443 |
| Significance | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 | <0.001 |

Values are Mean±SD of 6 rats in each group. Values in the parentheses are % increase (+) or decrease (-) compared to ethanol treated group) p-values: $^a$<0.001, $^b$<0.01 compared to control group; $^c$<0.001, $^d$<0.01 compared to 24 week ethanol treated group; $^e$<0.001, $^f$<0.01 compared to lecithin and ethanol treated group for 24 weeks. *nmole NADPH breakdown/min/mg protein; #µmole CDNB conjugate formed/min/mg protein; $^f$µmole $H_2O_2$decomposed/min/mg protein; $^{\Psi}$One unit of the enzyme was the amount of SOD capable of inhibiting by 50% the rate of NADH oxidation observed in the control. The specific activity was expressed as units/mg protein; $^{\omega}$nmole Pi/ /mg protein/h

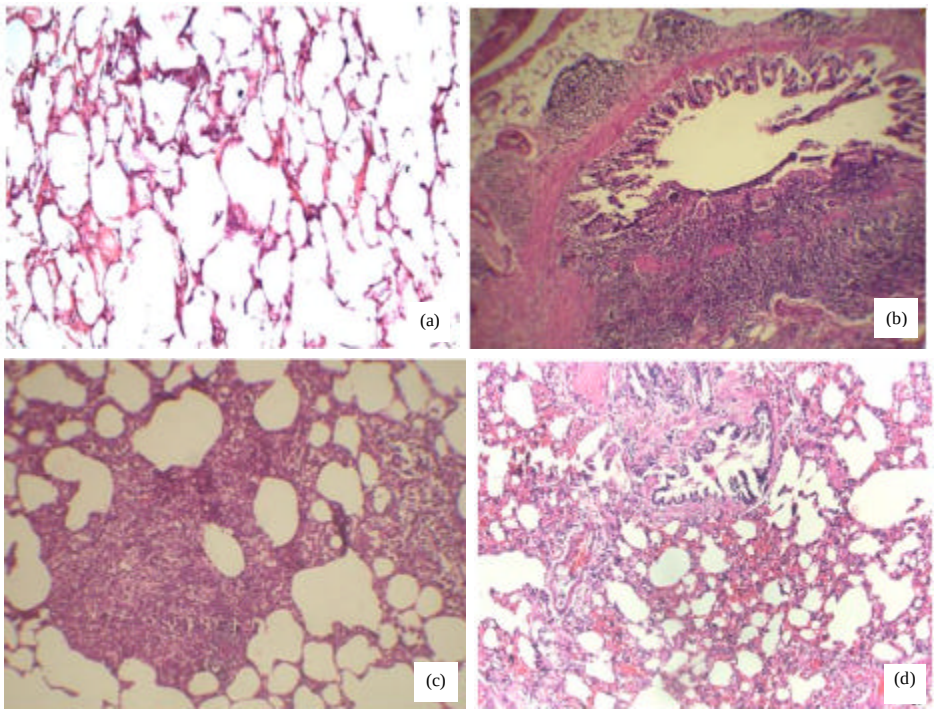


Fig. 3: Histological examination in different groups of animals exposed to different treatments. (a) Normal texture of lung in control animals,(b) Servere inflammation in ethanor treated lung, (c) lesithin treated rat-lung alveoli filled with inflammatory exudates and (d) Lecithin with Vitamin-B complex treated rat-with inflammatory exudates

## DISCUSSION

The intragastric ethanol infusion technique allowed maximal ethanol consumption and absolute control over ethanol-induced organ injury. In our model we administered ethanol (1.6 g/kg body weight/day) in the drinking water. This method has been used in multiple other studies in rats that have examined the effects of ethanol ingestion in a variety of tissues and physiological responses (Barrios *et al*., 1991; Carreras *et al*., 1992; Floyd *et al*., 1995; Uysal *et al*., 1985). The dose (1.6 g/kg body weight/day) used in this study, was based on our previous study (Das and Vasudevan, 2005, 2006a, b). Specially formulated isocaloric liquid diets were developed in the 1960s to study the hepatotoxicity of ethanol ingestion in animal models (Lieber, 1993). These diets were used because at the time it was unclear how much of an effect poor nutrition had in the development of cirrhosis, and there was some clinical bias that chronic alcohol abuse alone, in the absence of malnutrition, was not injurious. The consistent finding that ethanol ingestion produces similar hepatic injury regardless of the diet administered has solidified the evidence for ethanol and/or its metabolites as the cause of liver disease in chronic alcoholics (Holguin *et al*., 1998). Obviously, neither an isocaloric liquid diet with ethanol nor a diet in

which rats consume ethanol/water ad libitum as we used can be expected to reproduce the patterns of alcohol abuse and the sporadic dietary habits of patients with alcoholism (Holguin *et al*., 1998).

Rats which were consuming ethanol showed a lower increase in body weight due essentially to fat mass reduction (Das and Vasudevan, 2005). Ethanol being soluble both in water and lipids can diffuse rapidly through the mucous membrane of the oesophagous and stomach. After its absorption ethanol appears in both expired air and in urine. In addition, during alcohol ingestion, alcohol freely diffuses from the bronchial circulation directly through the ciliated epithelium where it vaporizes as it moves into the conducting airways. Some of vaporized ethanol can deposit back into the airway lining fluid and results in repeated exposure of the airway epithelium to high local concentrations of ethanol (Das, 2009).

Ethanol is not stored in the body, as whatever is ingested is oxidized (Antia, 1966; Eastwood and Passmore, 1986). When consumed in moderate amounts, the major part of the ethanol is metabolized primarily in the liver by cytosolic alcohol dehydrogenase (Cunningham and Bailey, 2001). However, ethanol metabolism through the cytochrome p-450 system in the lung is significant (Manatou *et al*., 1992) and may be sufficient to exert significant oxidative stress (Holguin *et al*., 1998; Moss *et al*., 2000), due to their unique structure and function (Lang *et al*., 2002).

Chronic ethanol exposure results in many pathophysiologic changes in cellular function caused by the ethanol itself and the effects of its metabolism (i.e., generation of acetaldehyde, Nicotinamide Adenine Dinucleotide (NADH), free radicals, and oxidative stress) (Polikandriotis *et al*., 2007). One of the proposed mechanisms of chronic ethanol induced-toxicity is the membrane damage due to the direct effect of lipid peroxidation products (Das and Vasudevan, 2005), i.e., TBARS, which was increased in the ethanol exposed rats in the present study (Lang *et al*., 2002). Protein nitration has been suggested to be a final product of highly reactive nitrogen oxide intermediates (e.g., peroxynitrite) formed in reactions between NO and oxygen-derived species such as superoxide. Nitrite, a stable metabolite of NO *in vivo* was increased in the lung homogenate of ethanol exposed rats in this study usually occurs through the activation of a constitutive Nitric Oxide Synthase (NOS) (Polikandriotis *et al*., 2007).

The tissue GSH concentration reflects its potential for detoxification and is critical in preserving the proper cellular redox balance for its role as a cellular protectant (Mari *et al*., 2001). Chronic ethanol ingestion decreased GSH in the lung tissue in this rat model, is in agreement with other observation (Holguin *et al*., 1998). Ethanol-induced GSH depletion may render lipids more susceptible to ROS attack. Alcohol can act as a prooxidant in tissues, including lung tissue (Nachiappan *et al*., 1994; Yang *et al*., 2002) and on lipids, including lung membrane lipids (Nachiappan *et al*., 1994; Manautou and Carlson, 1991). During the detoxification of lipid and other peroxides produced by free radical attack, glutathione peroxidase converts glutathione from a reduced state (GSH) to an oxidized one (GSSG) (Owens and Belcher, 1965). The NADPH dependent enzyme Glutathione Reductase (GR) converts GSSG back to GSH (Das and Vasudevan, 2005; Dinu and Zamfir, 1991). During an oxidative stress, there is flux of glutathione to the oxidized form, and the ratio of oxidized to reduced glutathione may be indication of this stress (Griffith, 1985). Decreased GSH level, increased GSSG level and redox ratio in this study indicated that the rats were suffering from oxidative stress due to chronic ethanol exposure.

Chronic ethanol ingestion resulted in significant decrease in GPx activity in the lung may be due to either free radical dependent inactivation of enzyme or depletion of its co-substrates i.e., GSH and NADPH (Das and Vasudevan, 2005, 2006b). Glutathione S-Transferase (GST) plays an essential role by eliminating toxic compounds by conjugating them with glutathione. Increased GST activity, and decreased GPx and GR activities, followed by thiol depletion are important factors in sustaining a pathogenic role for oxidative stress (Aniya and Daido, 1994; Das and Vasudevan, 2005, 2006b).

Chronic alcohol ingestion enhanced superoxide generation in the lung tissue (Polikandriotis *et al*., 2006) and the Superoxide Dismutase (SOD) dismutate these superoxide radical immediately (Das and Vasudevan, 2005). The cytochrome P450 2E1 was demonstrated to generate higher amounts of $H_2O_2$ (Nordsblom and Coon, 1977) and is linked to increased generation of hydroxyl radicals (Klein *et al*., 1983). Decreased SOD and catalase activities due to chronic ethanol exposure in the present study may be due to loss of NADPH, or generation of superoxide, or increased activity of lipid peroxidation or combination of all (Das and Vasudevan, 2005). Thus alcohol is capable of generating oxygen radicals, inhibiting glutathione synthesis, increasing malondialdehyde (MDA) levels and impairing antioxidant defense mechanisms in humans and experimental animals (Fernandez and Videla, 1981; Guidot and Roman, 2002; Speisky *et al*., 1985).

$Na^+$-$K^+$ ATPase participates in lung fluid clearance by exerting the active transport of sodium (Aytacoglu *et al*.,

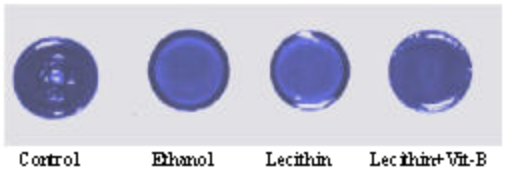


Fig. 4: Total MMP activity in the lung in different groups animals exposed to different treatment

2006). Oxidative stress plays a role in mediating the ethanol-induced down-regulation of lung Na`-K` ATPase (Rodrigo *et al*., 2002) as observed in this study (Table 2). Increased lipid peroxidation combined with decreased tissue Na`-K` ATPase activity may be associated with impairment of membrane phospholipids (Kolanjiappan *et al*., 2002). The decreased enzyme activity gives rise to the disintegration of the cells and consequently to the thickening of the air-blood barrier and alveolar degeneration (Aytacoglu *et al*., 2006), as observed histopthologically in the lungs of the ethanol exposed rats (Fig. 4). Evidences suggest that the increased reactive oxygen species due to ethanol intake causes phenotypic alterations in the lung (Polikandriotis *et al*., 2007) and stimulate the release and the formation of various inflammatory mediators (Polikandriotis *et al*., 2007; Calikoglu *et al*., 2003). These mediators lead to expression of endothelial adhesion molecules and vascular endothelial damage (Calikoglu *et al*., 2003).

It is also known that ultrastructural alterations could be triggered by changes in the intracellular redox state, which is reflected in either the depletion of thiols or in an increased number of ROS (Pimstone *et al*., 1971). Overall, chronic ethanol exposure increased lipid peroxidation and the redox ratio, decreased GSH level and Na`-K` ATPase activity, with altered antioxidant enzymes and the ultrastructural modifications in the lung of this study, is in agreement with other observation (Rodrigo *et al*., 2002).

This study have demonstrated that dietary supplementation with lecithin, a glutathione precursor decreased ethanol-induced oxidative stress, prevented activation of matrix metalloproteinases and inflammatory response and thereby limited lung injury. Another study concluded that some component of lecithin exerted a protective action against the fibrogenic effects of ethanol (Lieber *et al*., 1990). Though N-Acetylcysteine (NAC) is the only clinically approved glutathione precursor, a third study determined that treatment with the glutathione precursors S-adenosyl-L-methionine (SAM, 0.4 mg $mL^{-1}$) and NAC (0.163 mg $mL^{-1}$) given near the end of the period of ethanol ingestion could partially reduce the lung injury (Holguin *et al*., 1998). Evidence suggests that mitochondrial and not cytosolic glutathione depletion is the major defect in ethanol-induced hepatocellular dysfunction (Garcia-Ruiz *et al*., 1995; Fernandez-Checa *et al*., 1991). Both SAM and NAC were used because experience in models of ethanol-induced hepatotoxicity had shown that treatment with NAC alone does not increase hepatocyte mitochondrial glutathione levels in ethanol-fed rats (Fernandez-Checa *et al*., 1993), whereas supplementation with SAM may relate to the specific transport of cytosolic glutathione into the mitochondria (Holguin *et al*., 1998). However, the methionine pool is probably partially conserved at the expense of lecithin (Das and Vasudevan, 2006a) in this study. In addition, supplementation of trace amount of Vitamin-B complex with lecithin showed more protective effect in reversing the chronic ethanol-induced oxidative stress mediated effects. Vitamin-B complex is integrally involved in the metabolism of methionine and homocysteine. CBS requires Vitamin $B_6$ as a cofactor, whereas MS requires normal level of folate and Vitamin $B_{12}$ (Finkelstein, 2000).

Together, these findings persuasively argue that lecithin with Vitamin-B complex is not only a promising therapeutic approach in controlling ethanol induced immunomodulatory activities and liver damage processes (Das and Vasudevan, 2006a) but also in controlling ethanol-induced oxidative stress in the lung.

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