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Review Article

Ameliorating Effect of Essential Phospholipids Enriched with Virgin Coconut oil (Phoscoliv®) on Alcohol Induced Liver Toxicity: Possible Role in Oxidative Stress and Cellular Leakage

Ratheesh M[1*], Svenia P Jose[1], Sheethal S[1], Sandya S[2], Tariq Jagmag[3], Jayesh Tilwani[3], Anuradha Kulkarni[3]

[1]Department of Biochemistry, St. Thomas College, Kerala, India;[2]Department of Inorganic and Physical Chemistry, Indian Institute of Science, Bangalore, India;[3]Department of Research and Development, Mody Govt Pharmacy College, Mumbai, India

ABSTRACT

Background: Researchers had shown that essential phospholipids and virgin coconut oil are the very effective agents used for the treatment of several liver disorders. Thus, the current study aimed to evaluate the ameliorating effect of Phoscoliv (PL), a novel formulation using the combination of essential phospholipids enriched with virgin coconut oil.

Methods: In the study, adult wistar rats were grouped into three as Normal (N), Ethanol Treated (ET) (12.5 g/kg body weight of 90% [v/v]) and ET+ PL (0.5 mL/100g body weight) for 30 days. Chronic administration of ethanol induced severe damage to the liver which leads to the imbalance of normal cellular and metabolic activities. It was determined by evaluating the antioxidant status, liver marker enzymes, lipid peroxidation product, pro-inflammatory cytokines and histopathological analysis.

Results: Results revealed that the supplementation of PL enhance the antioxidants thereby reduced the oxidative stress associated liver damages by inhibiting lipid peroxidation product, pro-inflammatory cytokines release and leakage of liver marker enzymes. Thereby, improve the regeneration capacity of hepatic cells and maintain its normal functioning.

Conclusion: Hence, PL exhibited its potent hepatoprotective activity as well as antioxidant effect against alcohol induced liver toxicity.

Keywords: Antioxidants; Cytokines; Lipid peroxidation; Liver marker enzymes; Oxidative stress

INTRODUCTION

Liver is the second largest organ and heaviest gland in the human body. Main functional unit of the liver is known as hepatocytes and it perform a wide array of metabolic, secretory, and endocrine functions. Liver receives blood from both hepatic artery and hepatic portal vein. Through hepatic artery it receives oxygenated blood and through hepatic portal vein it obtains deoxygenated blood containing newly absorbed nutrients, drugs, and possibly microbes and toxins from the gastrointestinal tract. In current scenario, alcoholism and its consequences has become a most significant arising problem [1]. It is mainly due to the fact that the number of disease conditions and death by alcohol use is increasing day by day. Reports suggest that about 3.8% of global death and 4.6% of global disability is attributable to alcohol. During alcohol consumption, it affect almost all body organs, utmost liver is the most important, because liver is the major site of alcohol metabolism and can leads to Alcoholic Liver Disease (ALD), which found as the major risk factor for the development of liver diseases. About 10 to 35% of heavy drinkers may develop alcohol hepatitis which is an acute liver injury and severe condition may lead to death. It can act as a stimulant at lower doses [1,2].

ALD is a spectrum of clinical illness which is mainly characterized by the morphological changes of fatty liver to hepatic inflammation and necrosis (alcoholic hepatitis) to progressive fibrosis. Liver cirrhosis is a permanently damaged condition in which liver cells are replaced by scar tissue. So liver will be no longer to function and patient may die. Also, it associated with enhanced lipid peroxidation, protein modification, formation of the 1-hydroxyethyl radical and lipid radicals, and decreases in the hepatic antioxidant defense, particularly GSH levels. Generation of Reactive Oxygen

Correspondence to: Ratheesh M, Department of Biochemistry, St. Thomas College, Kerala, India, E-mail: biochemistrysstcp@gmail.com

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Species (ROS) such as superoxide, peroxide, and the hydroxyl radical are the most dangerous adverse effect of chronic alcohol consumption. Most of these ROS are converted to water instead of causing tissue damage. ROS can be toxic because they react with macromolecules such as protein, lipid, and DNA. They can induce lipid oxidation, inactivation of enzymes, mutations in the DNA destruction of cell membrane and ultimately cell [3]. During acute or chronic alcohol consumption the antioxidants become suppressed by the enhanced production of ROS results in a condition called oxidative stress. Oxidative stress is a state in which oxidation exceeds the antioxidant systems in the body secondary to a loss of the balance between them. Many biological processes such as apoptosis, viral proliferation, and inflammatory reactions can be influence by oxidative stress. Enhanced generation of ROS and the consequent oxidative stress can lead to ALD and other diseases such as cardiovascular diseases, atherosclerosis, various types of cancer, diabetes, neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease etc.

Several commercial drugs are available for the cure of liver diseases. But many of them may have side effects which may range from mild effect to severe health problems. Hence, it is necessary to find a better cure for liver diseases through natural products. An increasing number of studies in experimental animals put forward that dietary phospholipids might be of benefit in the treatment of ALD [4].This raises the possibility that synthetic or naturally occurring phospholipid isolates could be used as hepatoprotective nutraceuticals or functional foods. In the current study, we develop a novel and potent formulation, which consists of 40% of phosphatidylcholine and 60% virgin coconut oil. The aim of the present study was to evaluate the ameliorating effect of essential Phospholipids enriched with virgin coconut oil combination (hereinafter referred to as 'Phoscoliv®' (PL), patent pending registered product) on alcohol induced liver toxicity [5].

MATERIALS AND METHODS

Chemicals

Analytical grade chemicals were used for this study (Merck, Bangalore, India). RNA isolation kit and RT-PCR kit were purchased from Sigma-Aldrich, Bangalore, India. Liver function markers were analyzed using respective kits provided by M/s Agappe Diagnostics Pvt Ltd, Bangalore, India.

Preparation of virgin coconut oil

The solid endosperm of mature coconut was crushed, made into viscous slurry. The slurry was squeezed through cheese cloth to obtain coconut milk and refrigerated for 48 hours. After 48 h, the milk was subjected to mild heating (50^{o}C) in a thermostat oven. The obtained virgin coconut oil was filtered through cheesecloth and was used for the current study [5].

Preparation of phoscoliv

Phoscoliv is a formulation consists of 40% of phosphatidylcholine and 60% virgin coconut oil.

Study design and experimental protocol

Twenty-four rats were randomly divided into three groups each containing eight rats per group, as follows. Group I -Normal control rats (N); Group II – Ethanol treated rats (ET) (12.5 g/kg body weight of 90% [v/v]), Group III-Phoscoliv treated group (ET+PL) (0.5mL/100g body weight). Ethanol, PL, and distilled water (vehicle) were administrated by oral gavage (intragastrically) on every day morning after keeping them deprived of food for 10 hrs. After 30 days of study period, overnight fasted rats were sacrificed by cervical dislocation under an overdose of anaesthesia (Xylazine–Ketamine). Blood was collected by direct heart puncture into EDTA coated and non-EDTA vials for analysing serum biochemistry. Serum was separated from the clotted blood sample by centrifuging at 5,000 rpm for 10 min at 40C and was stored at -200 C for analyses. Protein was assayed by Lowry method. Samples of liver tissues from each group were taken and washed in PBS buffer and kept in 10% formalin for histopathological examinations [4,5].

Measurement of biochemical parameters in the serum

The levels of glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT) and Alkaline phosphatase (ALP) in rat serum were measured by a diagnostic kit from Agappa diagnostic Company, India.

Liver histopathological analysis

The entire liver tissue was rapidly dissected out and tissue sections (5μm thickness) fixed by immersion at room temperature in 10% formalin solution. For the histological examinations, paraffin-embedded tissue sections of liver tissue were stained with Hematoxylin–Eosin (H&E). The tissue samples were then examined and photographed under a light microscope for observation of structural abnormality. The severity of paw tissue inflammation was judged by two-independent observers blinded to the experimental protocol [6].

Statistical analysis

The results were analyzed using a statistical program SPSS/PC+, version 11.0 (SPSS Inc., Chicago, IL, USA). One-way ANOVA was employed for comparison test of significant differences among groups were determined. Pair fed comparisons between the groups was made by Duncan's multiple range tests. $P < 0.05$ was considered significant.

RESULTS

Effect of PL on endogenous antioxidant enzymes activity

Oxidative stress and the loss of hepatocytes activity were associated with chronic alcohol consumption generally reduced the antioxidant defence mechanism in the cirrhotic livers was evaluated by the antioxidant enzymes activities of SOD, CAT and GPx involved in the antioxidant defence mechanism. The activity of endogenous antioxidant enzymes such as SOD, CAT and GPx. In this study, SOD the hepatic primary antioxidant enzyme activity was significantly reduced in ethanol treated group compared to normal rats and these observations entailed the events of severe damage in the hepatocytes of liver cells of ethanol fed rats. On other hand, treatment with PL significantly ($P < 0.05$) increased the activity of SOD. Moreover, the activity of CAT was also decreased by ethanol consumption group as compared with normal group and was significantly increased by treatment with PL. Similarly, GPx activity was decreased significantly by ethanol treatment rats and was significantly increased by supplementation of PL as compared with ethanol treated group. This divergent result supported that the

treatment with PL might protect the hepatocytes from progressive damage against alcohol induced hepatotoxicity. Also, in ethanol treated rats, oxidative stress was in the peak and that can damage the hepatocytes severely. Treating the ethanol rats with PL significantly ($P < 0.05$) increased the level of endogenous antioxidant enzymes and induced the survival of hepatocytes [7].

Effect of PL on toxicity studies

The serum levels of SGPT, SGPT and ALP liver enzymes are important indicators of liver cell damage. The liver damage caused by ethanol treated rats significantly elevated the serum level of liver enzymes SGOT, SGPT and ALP as compared with normal rats. By PL supplementation significantly declined the level of these marker enzymes. These data demonstrated that the effects of toxicity caused by ethanol on the liver function could be effectively counterbalanced by PL administration [8].

Effect of PL on the GSH activity

In the ethanol treated group, there was a significant decrease in GSH content as there was a stress condition compared to normal group. By PL supplementation enhanced the GSH level.

Effect of PL on the concentration of TBARS level

The lipid peroxidation levels were measured in the liver tissue and MDA is an end product of lipid peroxidation, which has potent biological toxicity and can serious damage the structure of the cell membrane, leading to cell swelling and necrosis. The level of TBARS directly reflects the organ oxidative damage. Compared with that of control group, there was a significant increase in the TBARS level of ethanol induced rats. The administration of PL significantly ($p<0.05$) decreased in the lipid peroxidation production, thus reduced TBARS level [9].

Effect of PL on inflammatory cytokines

There was a significant elevation levels respectively in ethanol treated group when compared to normal rats. The elevated level of IL-6, significantly declined by PL administration [10].

DISCUSSION

The liver plays an essential role in the biological system that is responsible for the metabolism and clearance of drugs and xenobiotic, including ROS. However, when the amount of drugs or xenobiotic that is encountered has exceed the maximum metabolic capability of the liver; damaging effect of the toxins may lead to various liver disorders. Overconsumption of alcohol had been associated to a spectrum of liver injuries with varying degree of severity, with some common pathology including steatosis, foamy degeneration, steatonecrosis, venous lesion, and cirrhosis.

The liver enzyme markers like SGOT, SGPT and ALP are helpful to diagnose injuries in liver. During ethanol treatment there was an increase in the level of these liver marker enzymes and it indicates significant hepatocellular damage. These enzymes present in higher concentration in the cytoplasm of liver. During liver injury, enzymes will leak into the blood stream and their concentration will be high. During the administration of alcohol, structural changes will happen to the liver and an increase occurs in membrane permeability to ions and it causes translocation of SGPT and SGOT into blood stream. The level of serum ALP is related to the function of hepatic cell and increase in ALP is due to the presence of increasing biliary pressure. ALP also increased in ethanol administration and when PL supplementation significantly reduced the serum level of ALP.

Ethanol-induced liver injury is associated with increased oxidative stress and free radical-mediated tissue damage. Free radicals or ROS are responsible for ethanol induced oxidative stress. Free radicals formed from the ethanol mediated process have a great potential to react rapidly with lipids, which in turn leads to lipid peroxidation. The level of MDA has been widely used as a biomarker of LPO for many years. From the current study, there was a significant increase in TBARS level in ethanol treated group. On the other side, a significant decline in the level of TBARS on PL treated group shows the strong anti-inflammatory effect as well as prevents ROS generation.

Several studies have verified that enzymatic as well as non-enzymatic systems which maintaining cellular homeostasis are remarkably affected by alcohol in different models. In particular, the activities of SOD, CAT, GPx and GSH as well as the level of lipid peroxidation were changed in animals treated with alcohol. Antioxidant enzyme SOD helps to remove the superoxide's by converting it into Hydrogen peroxide. In the present study, ethanol administration enhances the liver injury leading to the failure of antioxidant defense mechanism and thereby occurs decrease in the level of antioxidant enzymes activity. When PL administrated rats showed significant elevated level of antioxidant enzymes such as SOD, CAT and GPx when compared with the normal rats. However, on supplementation of PL, increased the level of these antioxidant enzymes and thereby protecting the liver cells from further damage.

Another anti-oxidative compound is GSH; it is the main non-protein thiol component. GSH plays important role in enzymatic reactions therefore, participates in the regulation of the function and structure of cells. Alcohol consumption induces a decrease of the GSH reserve and, thus, causes a reduction of anti-oxidative properties. From other studies, they concluded that a reduction in the GSH more than 20% reduces the possibility of protecting cells against reactive oxygen species and causes damage to hepatocytes. Similarly, the specific result from our study stated that ethanolic rats had reduced GSH level but PL treatment influence to cause an increase in the GSH level.

Alcohol intake plays a pivotal role in the alterations of innate immune responses. The over consumption of alcohol by an individual have been reported to demonstrate a delayed and impaired hypersensitivity response. Alcohol is well known to alter cytokine levels in a variety of tissues including liver, plasma, lungs. Cytokines impact tissues in a complex manner that regulates inflammation, cell death, cell proliferation, cell migration, and healing mechanisms. These studies highlight the importance of examining cytokine changes across the time course of alcoholism to understand the initial changes directly resulting from alcohol use and the secondary pathology that occurs with late stage alcohol-induced tissue damage. Circulating cytokines such as tumor necrosis factor-alpha (TNF-alpha), interleukin-1 (IL-1) and IL-6 are found to be elevated in both chronic and acute alcohol-induced liver disease. These have been primarily correlated with the metabolic consequences and abnormalities of liver injury due to alcohol intake. The concentrations of all three cytokines have been correlated with biochemical parameters of liver injury, hepatic protein synthesis and serum IgG concentration. In the present study, the expression of inflammatory markers like IL-6

were up regulated in ET treated rats and it was significantly down regulated by PL treatment. Hence, PL shows an anti-inflammatory effect against chronic liver disease.

Histopathological study of rat liver supports above results. In the present study, the histopathology of the ethanol treated rats showed that the accumulation of fatty droplets, vesicular nuclei, dilated sinusoidal linings, hemorrhage (breakdown of blood vessels in the liver), cholestasis, inflammatory conditions as well as degeneration of hepatocytes. PL supplementation stimulates the regeneration of hepatocytes. Multinucleated hepatocytes were an evidence of regenerated liver hepatocytes. There was no hemorrhage; necrosis as well as inflammatory cells was observed. These results indicate that the hepatoprotective effect of PL on ethanol induced liver toxicity in rats.

CONCLUSION

These results suggest that PL can inhibit liver injury due to ethanol administration and, thereby inhibits the pathogenesis liver tissue and protecting it from further oxidative stress and damage.

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