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Hepatoprotective effect of essential phospholipids enriched with virgin coconut oil (Phoscoliv) on paracetamol-induced liver toxicity

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Abstract

The prevalence of liver disease is increasing year by year and it is recognized as a main health burden across the world. Nowadays, dietary nutraceuticals are found to be very effective in the prevention and treatment of liver diseases. The virgin coconut oil and phosphatidylcholine are found to have a wide range of therapeutic efficacy and the most important among them is its hepatoprotective activity. In the present study, we had evaluated the hepatoprotective effect of the novel formulation with the combination of these two which is named as Phoscoliv. For the study, adult Wistar rats were grouped into Normal control, Paracetamol-treated, and Paracetamol along with Phoscoliv-treated group. In order to evaluate the hepatoprotective effect of the drug, various parameters were analyzed. Data obtained from the study showed that Phoscoliv supplementation were found to significantly boost up the antioxidant status by enhancing the SOD, CAT, GPx, and GSH level and thereby inhibit the generation of ROS and also blocked lipid peroxidation, which was confirmed by the reduced level of TBARS. The release of pro-inflammatory cytokines was also decreased, which was eventually helped to maintain the normal architecture of the liver. Thus, from the overall result of this study reveals that Phoscoliv can be effectively used as a potent and safe hepatoprotective medicine.

Practical applications

The over or unwanted usage of synthetic medicine is a serious problem because it can cause so many adverse health effects. Liver-related disorders are the major side effects of these drugs. Food habits of ancient people dictate that there is no other better medicine than a good food. So, treating a disease with a food or compounds derived from a food item will be more effective. Virgin coconut oil is a type of natural and organic oil, which has the capability of maintaining the body in a healthy state. Likewise, phosphatidylcholines are very important phospholipid nutrients necessary to keep the cells healthy. Both these have the potential to protect and prevent the liver damages. Therefore, the combination of these two can exhibit profound hepatoprotective activity.

KEYWORDS
antioxidants, cytokines, hepatotoxicity, lipid peroxidation, paracetamol, Phoscoliv

1 | INTRODUCTION

Virgin coconut oil is a naturally processed oil, obtained either from coconut kernel or coconut milk, which is one of the important sources of dietary fat. It is processed using a low heat process and its nutritional value and health benefits have been recognized for many years. So, it has a significant role in our diet (Kamariah et al., 2008). There are lots of scientific reports supporting the pharmaceutical properties of virgin coconut oil as compared to other oils. This includes its hepatoprotective, anti-inflammatory, antimicrobial, anti-hypercholesterol effect, and moreover, it helps to improve the antioxidant status and control the lipid peroxidation process (German & Dillard, 2004; Nevin & Rajamohan, 2004; Van Immerseel et al., 2004). Therefore, virgin coconut oil gains more acceptance because of its nutraceutical and pharmaceutical properties.

All the beneficial or detrimental effects resulting from the consumption of food, drinks, or drugs will ultimately reflect in the cells and cellular activities. So, maintaining the cells in a healthier condition is very crucial to get rid of many kinds of diseases. Phosphatidylcholine is a phospholipid which is considered as one of the major building blocks and essential nutrient for the cells (Vance & Ridgway, 1988). It gains utmost importance among the researchers as it possesses various properties like antioxidant, anti-inflammatory, hepatoprotection, anti-fibrotic etc. (Akin et al., 2007; Chung et al., 2014). There are lots of previous studies showing the beneficial effects and hepatoprotective activity of phospholipids isolated from various plants and animals (Bubnova & Aronov, 2014; Hassanein et al., 2011; Li et al., 2018; Yang, 2017).

The hepatoprotective properties of phosphatidylcholine are mainly based on the membrane-dependent activities, specifically, maintaining the cellular integrity, regeneration, and protection of the damaged cells, controlling the metabolic processes that take place in between the intracellular and intercellular region by being involved in the passage of different molecules through the membranes (especially in the hepatocytes), stimulating the activation of membrane bound proteins like lecithin cholesterol acyltransferase, lipoprotein lipase, cytochrome oxidase etc., and possessing bounded polyunsaturated fatty acids which are the precursors of certain cytoprotectants such as eicosanoids which will be released as required (Gundermann, 1993; Kuntz, 1995). The ability of phosphatidylcholine to dissolve the fats accumulated in the liver will be very effective for the people having fatty liver (Fricker, 2010; Zeisel et al., 1991). Therefore, it is inevitable but the endogenous production of phosphatidylcholine is not enough to meet the normal bodily activities. So, it is very necessary to include it in our regular diet, the deficiency can adversely affect the normal functioning of liver.

The liver is one of the most important organs which plays a significant role in maintaining the homeostasis of the body by performing the functions like metabolism, secretion, storage, and detoxification (Maheswari et al., 2008). As a part of the detoxification process, the liver is continually exposed to various xenobiotics, chemotherapeutic agents, or other pollutants from the environment. Therefore, the liver is more susceptible to toxic hepatic injuries and infections. In

recent years, hepatic diseases are becoming a global issue and the number of patients are also increasing (Francesca, 2012). The major contributing factor of hepatotoxicity is either due to the adverse side effect caused by the use of conventional drugs or the overdose of certain analgesic drugs like paracetamol, which is commonly known as drug-induced hepatotoxicity (Arhoghro et al., 2009; Tanne, 2006).

Paracetamol (PCM) or acetaminophen is a very commonly used analgesic or antipyretic drug (Cover & Mansouni, 2005). In the therapeutic dosage of PCM, it will rapidly metabolize in the liver and majority is eliminated by conjugation to nontoxic sulfated and glucuronidated metabolites and a small percentage of PCM will undergo oxidation with the help of cytochrome p-450 enzyme resulting in the formation of highly reactive N-acetyl-p-benzoquinonimine (NAPQI) (Bunchorntavakul & Reddy, 2013; McGill & Jaeschke, 2013). In the normal process of detoxification, this reactive NAPQI is detoxified by the antioxidant enzyme glutathione (GSH) and always maintains an equilibrium to neutralize the toxic environment (Song et al., 2014). However, overdose can lead to the excessive production of NAPQI that can inflict a shift in this equilibrium due to the saturation of sulfation and glucuronidation process and depletion of GSH, which leads to create an oxidative stress condition (Dai et al., 2006). As a result, progressively more reactive oxygen species (ROS) will be released and that will trigger the cellular necrosis by organelle swelling along with membrane dysfunction through disruption of the mitochondrial membrane permeability transition pores (Yuan & Kaplowitz, 2013).

In addition to this, drug-induced hepatotoxicity is often allied with an inflammatory response which will significantly intensify the severity of liver injury (Rolando et al., 2000). Impairment in the liver function has harmful effects on the other organs too because of the toxic factors released from damaged liver and also due to the loss of detoxifying pathways (Ferenci, 2002). Therefore, by considering the lack of reliability of modern medicines and the rigorousness of the liver diseases, the exploration of a novel and potent formulation is very essential. Moreover, the efficacy and safety of naturally derived food products are starting to get the attention of an increasing number of researchers and pharmaceuticals (Xie, 2016).

Thus, the aim of the present study was to evaluate the hepatoprotective effect of Phospholipid–virgin coconut oil combination (hereinafter referred to as "Phoscoliv," patent pending and registered formulation) on PCM-induced liver toxicity. So, we assume that the combination of phospholipids and Virgin coconut oil act synergistically and bring out a strong hepatoprotective effect.

2 | MATERIALS AND METHODS

2.1 | Chemicals

All the chemicals used for the study were high quality analytical grade reagents. SGOT, SGPT, and ALP kits were obtained from M/S Agappe diagnostics Company India Pvt Ltd. and ELISA Kits were purchased from sigma-Aldrich, USA.

2.2 | Preparation of Phscoliv

Phscoliv is a novel formulation which consists of phosphatidylcholine and virgin coconut oil. Virgin coconut oil is prepared from the solid endosperm of mature coconut. It was crushed and made into a viscous slurry. The slurry was then squeezed through cheese cloth to obtain coconut milk and it was refrigerated for 48 hr. After 48 hr, the milk was subjected to mild heating (50°C) in a thermostat oven. The obtained virgin coconut oil was filtered through cheese cloth (Nevin & Rajamohan, 2006).

For the preparation of Phscoliv, different ratios of phosphatidylcholine and virgin coconut oil were selected. Each composition with different ratios were mixed thoroughly for 3 hr using a shaker and then, it was filtered through cheese cloth. Again the mixing process was repeated for 1 hr and strained out. From the screening study, it was found that, phosphatidylcholine and virgin coconut oil in the ratio 40: 60 showed potent hepatoprotective effect by evaluating the liver marker enzymes (Data not shown). Hence, this ratio was used for the present study.

2.3 | Animals

Healthy adult male Wistar rats (weighing 150–200 g) bred in the host department animal facility were used for this study. They were kept under a controlled environment, temperature (24°C–26°C), humidity (55%–60%), and photoperiod (12:12 hr light—dark cycle). The rats were housed in the cages containing sterile paddy husk as bedding material throughout the experiment and the commercial laboratory balanced diet (Amrut laboratory Animal feeds, Maharashtra, India) and tap water were provided ad libitum. The animals received humane care, in compliance with the host institutional animal ethics guidelines. All experiments were conducted as per the guidelines of the animal ethics committee CPCSEA (Registration No. CAF/361/2015) according to Government of India accepted principles for laboratory animals' use and care.

2.4 | Study design and experimental protocol

For the study rats were divided into three groups and each group contain six rats.

Group I served as Normal control rats (N)

Group II served as Paracetamol-treated rats (PCM) (750 mg/Kg b.wt.)

Group III served as Phscoliv-treated group (PCM+PL) (0.5 ml/100 g b.wt.)

Each day Group II and III received a single dose of 750 mg/Kg b.wt. Paracetamol (Uchendu et al., 2018) and Group III alone received a single dose of 0.5 ml/100 g b.wt. Phscoliv, administered through intragastrically [Dose was selected from preliminary screening study (Data not shown)].

At the end of 30 days of experiment, blood sample of each rat was collected from retro orbital plexus into a sterile tube for separating serum for the estimation of biochemical parameters. Rats were then sacrificed by euthanasia and liver tissue was dissected out. For histological analysis, a portion of the liver tissue was transferred into 10% buffered formalin and another portion was used for the preparation of tissue homogenate.

2.4.1 | Estimation of antioxidant activity

Assay for the estimation of catalase (CAT) activity was determined using the method of Maehly and Chance (1954). The activity of glutathione peroxidase (GPx) was determined by the method of Lawrence and Burk (1976) as modified by Agergaard and Jensen (1982). Superoxide dismutase (SOD) activity was determined using the method described by Kakkar et al. (1984). The enzyme activity was evaluated in liver tissue homogenate and its activity is expressed in unit/mg proteins.

2.4.2 | Estimation of glutathione content (GSH)

In liver homogenate GSH content was estimated according to the method of Benke and Murphy (1974).

2.4.3 | Estimation of malondialdehyde (MDA)

Liver tissue was homogenized in phosphate buffer (0.12M) pH 7.5, centrifuged, and the supernatant was used for the estimation of MDA by the method of Ohkawa (1979).

2.4.4 | Estimation of SGOT, SGPT, and ALP

The serum levels of Serum glutamic pyruvic transaminase (SGPT), Serum glutamic oxaloacetic transaminase (SGOT), and Alkaline phosphatase (ALP) were measured using the diagnostic kits from M/S Agappe diagnostics Company, India.

2.4.5 | Estimation of pro-inflammatory cytokines

The serum level of cytokines was determined by enzyme-linked immunosorbent assays (ELISA). IL-6 and TNF-α were measured by following the instruction of manufactures (Sigma-Aldrich, USA).

2.4.6 | Histopathological analysis of liver tissue

The entire liver tissue was rapidly dissected out and tissue sections (5μm) 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 liver tissue inflammation was judged by two-independent observers blinded to the experimental protocol. The degree and severity of hepatocellular changes were scored based on the grading system done by El-Beshbishy et al. (2010). The severity of various features of hepatic injury was evaluated based on those following scoring schemes: (–) Normal, (+) Mild effect, (++) Moderate effect, and (+++) Severe effect.

2.4.7 | Assay of protein

Protein in liver homogenate was assayed by the method of Lowry et al. (1951).

2.4.8 | 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 were made by Duncan's multiple range tests. $p < .05$ was considered significant.

3 | RESULTS

3.1 | Effect of PL on antioxidant enzymes activity

The protective effect of PL against the severe oxidative stress induced by PCM was measured by evaluating the activity of the antioxidant enzymes. SOD, CAT, and GPx are the major free radical scavengers which were reduced significantly in PCM-treated rats as compared to the normal control rats. This indicates that there was an excessive amount of ROS generation by PCM administration. In contrast, treatment of rats with PL significantly ($p < .05$) increased the activity of SOD, CAT, and GPx as similar to normal control group. Result is shown in the Figure 1.

3.2 | Effect of PL on hepatocellular antioxidant enzyme GSH

The GSH activity was depleted significantly ($p < .05$) in the liver tissue of PCM-treated rats as compared to the control group. However, the treatment with PL showed maximum restoration of GSH and enhance the GSH status in the body (Figure 2).

3.3 | Effect of PL on the levels of SGOT, SGPT, and ALP

The serum SGPT, SGOT, and ALP in the rats treated with PCM was found to be significantly elevated ($p < .05$) and it was the indication of severe liver necrosis. However, it was noticed that the level of
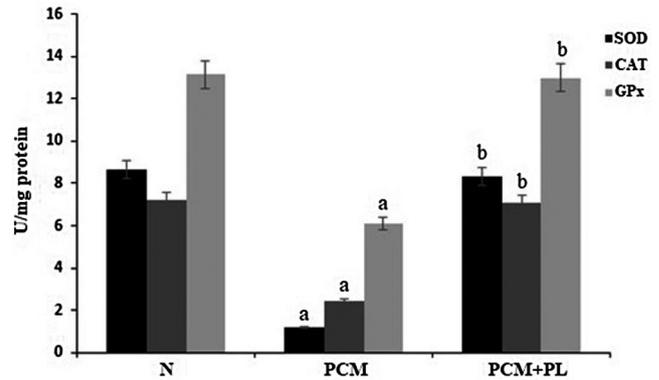

FIGURE 1 Effect of PL on the enzymatic antioxidant activities of SOD, CAT, and GPx enzymes. The values expressed as average of six samples ± SD in each group. "a"-Statistical difference with normal group at $p \leq .05$. "b"-Statistical difference with PCM-treated rats at $p \leq .05$. Catalase, U-µmol H_2O_2 decomposed/min; GPx, U-µmol NADPH oxidized/min; SOD, U-enzyme concentration required to inhibit chromogen production by 50% in 1 min
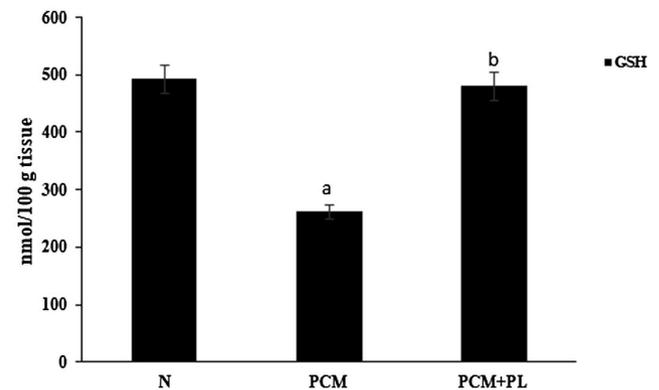

FIGURE 2 Effect of PL on GSH level. Values expressed as average of six samples ± SD in each group. "a"-Statistical difference with normal group at $p \leq .05$. "b"-Statistical difference with PCM-treated rats at $p \leq .05$.

these liver marker enzymes was maintained almost closer to normal by the administration of PL. Hence, it clearly showed the protective effect of PL over the liver enzyme leakage due to the PCM induction. Results are shown in the Figure 3.

3.4 | Effect of PL on lipid peroxidation end product TBARS

The result shows that the administration of PCM leads to an increased generation of free radicals thus, immense lipid peroxidation process was taking place. As a result, the level of TBARS was significantly increased which led to the deterioration of hepatocyte as compared to the normal control. In contrast, the group treated with PCM along with PL significantly ($p < .05$) reduced the TBARS level (Figure 4).
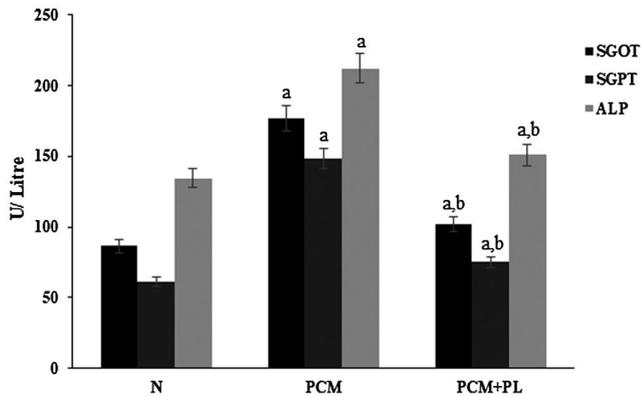
FIGURE 3 Effect of PL on liver enzyme markers. The values are expressed as mean $\pm$ SD of six rats in each group. a-Statistical difference with control group at $p < .05$. b-Statistical difference with PCM-treated rats at $p < .05$. U: SGOT-μmol of oxaloacetate liberated /min/mg protein; SGPT-μmol of pyruvate formed/min/mg protein; ALP-amount of enzyme to decompose 1 μmole of P-NPP/ minute at 25°C
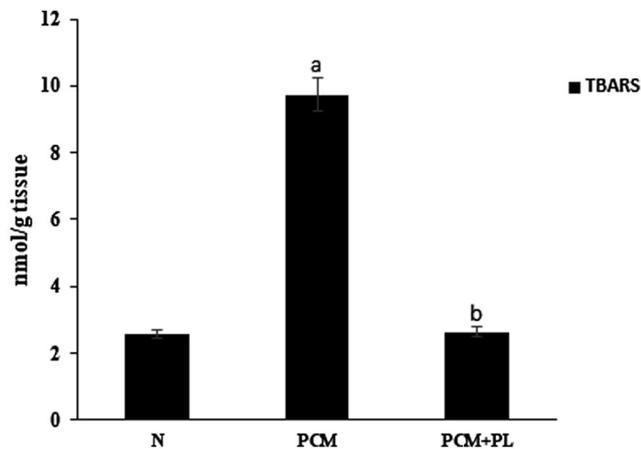

FIGURE 4 Effect of PL on TBARS level. Values expressed as average of six samples $\pm$ SD in each group. "a"-Statistical difference with normal group at $p \leq .05$. "b"-Statistical difference with PCM-treated rats at $p \leq .05$

3.5 | Effect of PL on cytokines like IL-6 and TNF- α

Inflammatory condition developed as a result of hepatic toxic injury was studied by measuring the pro-inflammatory cytokines like IL-6 and TNF-α. They are released into the bloodstream both from the liver and other distal sites. The results also indicated that PCM-treated control group showed elevated levels of IL-6 and TNF-α in comparison to the normal control. However, when comparing PL with PCM control, the release of excess pro-inflammatory cytokines was significantly ($p < .05$) blocked (Figure 5).

3.6 | Histopathological examination of liver tissue

Table 1 shows the histological scoring of liver tissue. From the stained section of liver tissue shows that the normal control group had
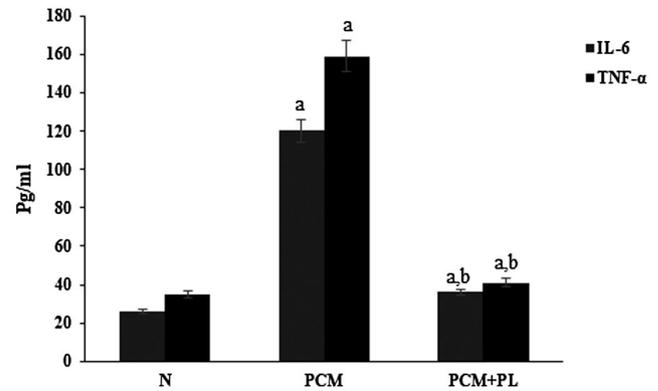

FIGURE 5 Effect of PL on pro-inflammatory cytokines. Values expressed as average of six samples $\pm$ SD in each group. "a"-Statistical difference with normal group at $p \leq .05$. "b"-Statistical difference with PCM-treated rats at $p \leq .05$

well-defined structure and arrangements of hepatic cells whereas, PCM-treated group showed degenerative changes characterized by loss of tissue architecture with cells showing granular cytoplasm and section also shows inflammatory cell infiltrates and liver steatosis. The rats treated with PL showed its effect on liver protection against the drug. PL maintained the tissue architecture almost similar to the normal liver tissue and protected from steatosis and severe infiltration of inflammatory cells (Figure 6).

4 | DISCUSSION

Drug-induced hepatotoxicity is one of the major reasons for the mortality and morbidity of human beings across the world (Bhawna & Kumar, 2009). The common xenobiotic-induced experimental model used for the screening of the hepatoprotective effect of various drugs is PCM-induced liver injury (Boyd & Bereczky, 1966). At higher doses, PCM causes hepatic necrosis by increasing the amount of reactive NAPQI and this will bind to the biomolecules and attack them (Ramachandra et al., 2007). PCM is also directly involved in the induction of oxidative stress resulting in lipid peroxidation, depletion in antioxidants, and ATP synthesis, and ultimately leading to liver damage (Rabiul et al., 2011).

In order to prevent the cellular damage due to the excessive production of NAPQI, the well-known antioxidant GSH plays a key role. It will act against NAPQI and as a part of it GSH will be oxidized to GSSG (GSH disulfide). Consequently, the level of GSH in the liver will steadily go down, which results in severe oxidative stress (Kerksick & Willoughby, 2005; Yanpallewar et al., 2003). It has been shown that to keep up the GSH from being depleted, it is very important to provide direct protection against PCM-induced hepatotoxicity (Ahmed & Khater, 2001). Our study also reveals the same, that is, PCM treatment reduced the GSH level significantly in the liver tissue, but the supplementation of PL strongly helps to enhance the GSH level.

TABLE 1 Histological scoring of liver tissue

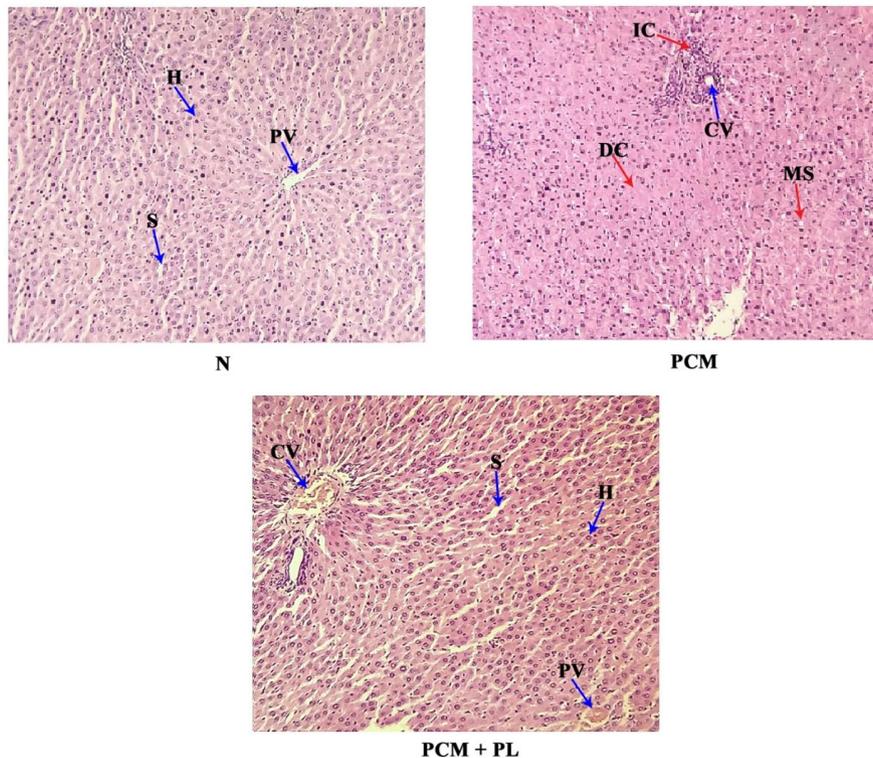| Treatments	Doses	Steatosis	Necrosis	Inflammation
N	–	–	–	–
PCM	750 mg/Kg b.wt.	++	+++	+++
PCM+PL	0.5 mL/100g b.wt.	–	–	+

FIGURE 6 Histopathological examination of liver tissue (H&E stain) (40X). Abbreviations: CV, central vein; DC, degenerated cells; H, hepatocytes; IC, inflammatory cells; MS, microsteatosis; PV, portal vein; S, sinusoids

PCM not only depleted the GSH antioxidant, but also affect the other antioxidants in the body. The same condition of GSH was also observed in the case of enzymatic antioxidants like SOD, CAT, and GPx. These are the antioxidants which play a significant role as the natural scavenger system and help to prevent the body, mainly from the superoxide and hydrogen peroxide radicals and act as a shield for cellular components against oxidative stress (Scott et al., 1991). During the induction of PCM there will be an imbalance between ROS generation and antioxidant defense, which will result in stress and cellular damage (Gini & Muraleedhara, 2010). In this study, it was observed that there was a significant increase in the activities of SOD, CAT, and GPx in PL-treated rats. This showed that PL can reduce the oxidative damage to the tissues besides improving the activity of hepatic antioxidant enzymes. Hence it is due to the ability of PL to interact with hydroxyl, superoxide, alkoxyl, and peroxyl radicals that is able to scavenge them. This indicates that PL has the potential to protect the liver from oxidative stress induced by drug-mediated liver toxicity.

Studies have shown that the reduction in antioxidant status is also associated with the elevated level of TBARS produced as a result of lipid peroxidation (LPO) (Shao, 2008). It is a kind of destructive process by which polyunsaturated fatty acids in cellular membranes undergo reaction with oxygen, resulting in the formation MDA and other compounds (Muriel et al., 1992). LPO disturbs the membrane potential, which will lead to tissue necrosis. It was clearly observed that there was a significant increase in TBARS level in liver tissue intoxicated with PCM and reduced by the administration of PL. This shows that due to its strong antioxidant action, LPO is inhibited and maintain the membranes from free radical attack.

All the changes inflicted by PCM finally end in toxic injuries that become the causative factor for the activation of Kupffer cells and adjacent sinusoidal endothelial cells to secrete different mediators, including pro-inflammatory cytokines like TNFα, IL6 along with ROS (Laskin & Laskin, 2001). These pro-inflammatory cytokines can induce apoptosis and impairment in cellular function by amplifying the inflammatory processes and induce damage to the hepatocytes and other hepatic cells which further creates a chronic inflammatory condition (Lacour et al., 2005). In our study, we also noticed that these pro-inflammatory cytokines in PCM-treated rats were very high, but PL interrupts the release of cytokines and protect the hepatocytes from inflammatory mediators.

Liver damage was confirmed by estimating the levels of SGOT, SGPT, and ALP. These are the liver enzyme markers which are commonly used to diagnose liver injuries. By the excessive intake of drugs like PCM, these enzymes will increase significantly due to the hepatocellular injury and as a result, the enzymes will leak into the bloodstream and their concentration will be hiked (Nkosi et al., 2005). Elevated levels of liver marker enzymes were found that PCM-treated rats due to the extensive liver damage induced by the drug. We found that PL administration reduced the concentrations of SGOT, SGPT, and ALP levels. Therefore, it is clear that PL helps to prevent the leakage of marker enzymes by protecting the liver tissues and cellular constituents normally.

The liver is constantly metabolizing drugs and other xenobiotics, so it is very much prone to drug-induced damages. The ultimate effect of all the toxic mechanism can be seen in the histological study of liver tissue. The hepatoprotective effect of any substance is mainly dependent on its ability to restore the normal histology of liver or to reduce the toxic effects induced by the external factors. In the present study, liver section treated with PCM displayed the severity and intensity of the over dosage of such kind of drugs over the normal architecture of liver. It may be due to the overproduction of reactive free radicals which mediate the tissue damage. Along with the negative side effects of PCM intoxication, we found the competency of PL to protect the liver from drug-induced toxicity. PL helps to boost up the survival and regeneration of hepatocytes and maintain the liver cells in their normal state without leading to the different stages of liver damage. Thus, the hepatoprotective effect of PL against drug-induced liver toxicity was clearly visible in the histopathological assessment.

5 | CONCLUSION

The results obtained from the present study shows that, Phoscoliv, the combination of phosphatidylcholine and virgin coconut oil prevented the drug-induced hepatotoxicity via free radical scavenging, reducing inflammation as well as cellular leakage and thereby reverted back to the normal liver morphology. So, Phoscoliv will be a better solution to the people who are suffering from hepatic damage.

CONFLICT OF INTEREST

All the authors are disclosing the conflict of interest. Phoscoliv is a patent pending registered product of Glowderma Pvt. Ltd. Mumbai.

AUTHOR CONTRIBUTION

Sheethal Sreevallabhan: Data curation; Formal analysis; Writing-original draft. **Ratheesh Mohanan:** Conceptualization; Methodology; Supervision; Writing-review & editing. **Svenia P Jose:** Investigation; Software; Validation. **Sandya Sukumaran:** Methodology; Project administration; Writing-review & editing. **Tariq Jagmag:** Funding acquisition; Writing-review & editing. **Jayesh Tilwani:** Resources; Visualization. **Anuradha Kulkarni:** Writing-review & editing.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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