Research Article

Differential Role Of Prkcε In The Pathogenesis Of Chronic Alcohol-Induced Liver Injury

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Abstract

Chronic alcohol consumption is a major cause of liver injury, progressing from hepatic steatosis to inflammation, necrosis, and fibrosis. Protein kinase C epsilon (PRKCε) has been implicated in alcohol-induced hepatic steatosis; however, its contribution to subsequent inflammatory and necrotic injury remains unclear. The present study investigated the differential role of PRKCε in chronic ethanol-induced liver injury using an experimental animal model. Mice were maintained on a high-fat diet and treated with either saline or PRKCε antisense oligonucleotides (ASO) for four weeks, followed by ethanol exposure. Hepatic lipid accumulation, fatty acid synthase (FAS), tumor necrosis factor-alpha (TNF-α), plasminogen activator inhibitor-1 (PAI-1), inflammation, necrosis, and fibrin deposition were evaluated. PRKCε ASO treatment significantly attenuated ethanol-induced hepatic lipid accumulation and prevented the ethanol-mediated increase in FAS expression. Furthermore, PRKCε inhibition completely suppressed the elevation of TNF-α expression associated with ethanol exposure. Despite these effects, PRKCε ASO administration did not significantly reduce chronic ethanol-induced hepatic inflammation or necrosis. Similarly, the ethanol-induced upregulation of PAI-1 and accumulation of fibrin were unaffected by PRKCε inhibition. These findings indicate that PRKCε plays an important role in alcohol-induced hepatic steatosis and associated metabolic alterations but is not the primary mediator of chronic inflammatory and necrotic liver injury. The persistence of PAI-1 elevation and fibrin deposition despite PRKCε inhibition suggests that these pathways may independently contribute to progression of alcohol-induced hepatic damage. Thus, targeting multiple pathological pathways may provide a more effective strategy for preventing chronic alcohol-related liver injury.

Keywords: PRKCε; Protein kinase C epsilon; Chronic alcohol consumption; Ethanol-induced liver injury; Hepatotoxicity.

Introduction

Historically, the lack of a standard effective treatment for alcoholic liver disease (ALD) stems from a poor understanding of the disease process. Today, it is estimated that millions die from ALD each year worldwide, yet scientific research into ALD has significantly improved our knowledge of all phases of ALD pathology [1,2]. Steatosis represents one of the four major pathological stages of ALD. Until very recently, it has been widely assumed that steatosis would not have a negative effect on individual cases of ALD, but now research indicates that preventing the development of steatosis may slow down the progression of disease [3,4]. Importantly, some evidence suggests that inhibiting the development of steatosis would also slow the advancement to the later, more critical stages of ALD. Research has also provided evidence to support the idea that stimulating the PRKCε signalling pathway may ultimately be the most effective way to treat ALD because it induces steatosis in patients with NAFLD [5]. Gandapur et al. (2026) suggest that if liver steatosis is favored by the oxidation of free fatty acids, then diacylglycerol (DAG) is likely to serve as a source of fatty acids. Therefore, it appears that PRKCε may be an important pathway in both ALD and NAFLD [6]. Both in the acute phase of the administration of EtOH and after long-term administration of EtOH, this model shows minimal or no inflammation or necrosis in the liver. In contrast, many other forms of inflammation (e.g., lipopolysaccharide) would provide data to support that PRKCε is implicated in the later stages of chronic alcohol-induced steatosis [7,8]. By decreasing the formation of alcohol-induced steatosis, more progress in ALD may be halted. Additional studies are warranted to validate the possible role of PRKCε in progressing later stages of alcohol-induced liver disease. Therefore, the study will evaluate the potential contribution of PRKCε to chronic EtOH-induced liver injury.

Materials and Method

Animal Procurement and Housing

The animal experimental studies were conducted at Systemic Life Sciences & Research Pvt. Ltd. Preclinical Research Facility, Aushapur, Medchal-Malkajgiri District, Telangana, India, in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA). The experimental protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of Systemic Life Sciences & Research Pvt. Ltd. under IAEC approval number 31/IAEC-III/SLSRPL/2025, dated 26 November 2025. The approved protocol included 18 C57BL/6 mice and was authorized for a period of 12 months**.** Eight-week-old male C57BL/6 mice were procured from an approved laboratory animal supplier and housed under standard environmental conditions in the pathogen-controlled preclinical research facility of SLSRPL. Animals were allowed free access to standard laboratory feed and tap water throughout the acclimatization and experimental periods, except where otherwise specified by the experimental protocol. An intragastric cannula was surgically implanted in the animals, and enteral feeding was subsequently performed according to the established experimental procedure. PRKCε antisense oligonucleotide (PRKCε-ASO) was procured from Integrated DNA Technologies (IDT), Bengaluru, India. PRKCε-ASO was administered intraperitoneally at a dose of 25 mg/kg twice weekly, according to the approved experimental protocol. Appropriate control treatments were administered to the respective control groups. At the completion of the experimental period, animals were anesthetized using ketamine/xylazine (100/15 mg/kg, intramuscularly) before sacrifice. Blood was collected from the vena cava immediately prior to sacrifice, and citrated plasma was separated and stored at −80 °C until further analysis. Liver tissues were collected immediately after sacrifice. Portions intended for histopathological examination were preserved in 10% neutral buffered formalin, whereas other liver samples were immediately snap-frozen in liquid nitrogen and stored appropriately for subsequent biochemical and molecular analyses. All procedures involving experimental animals were performed at Systemic Life Sciences & Research Pvt. Ltd. in accordance with the approved IAEC protocol, CPCSEA guidelines, and applicable standards for the ethical care and use of laboratory animals.

Clinical Histological Analysis

Hematoxylin and eosin stains were used to assess pathology after chronic enteral alcohol feeding and were cut from paraffin-embedded, formalin-fixed liver specimens (6 µm). The pathological scoring system was used [9]. Micro- and macro-vesicular fat drops were defined in terms of less than or more than 50% total cell volume, respectively. Standard kits were used to measure plasma levels of ALT and AST. Daily urine samples were collected, and urine alcohol levels were determined spectrophotometrically using standard methods. Chloroacetate esterase activity (CAE) was measured by staining liver sections with a naphthol AS-D CAE kit for mapping the accumulation of neutrophils. Quantitative immunofluorometric measurements of the accumulation of fibrin matrices were performed using image analysis. Hepatic lipids were stained using oil red O, and counterstained with hematoxylin, using image analysis for quantification.

Biochemical Analyses

As previously mentioned, Western blotting for PRKCε was carried out. The procedure of RNA extraction and real-time reverse transcriptase PCR was conducted in the same manner as previously described [7,10]. The extraction of hepatic lipids was completed using a combination of methanol and chloroform in aqueous phase for the determination of the level of hepatic lipids. Mass spectroscopy was employed for the measurement of DAG, and its level was expressed in terms of mg wet weight of the liver tissue. A standard kit was used for the colorimetric determination of non-esterified fatty acids. The Cobas Mira chemical analyzer with properly calibrated standards was used for the colorimetric measurement of phospholipids, cholesterol, and triglycerides. Before extraction, the Bradford test was conducted to bring the values of lipids like cholesterol and triglycerides to protein in the homogenate.

Statistical Analyses

The outcome was presented as means SEM. The significance of the difference between treated groups was established using ANOVA, followed by Bonferroni's test. The Mann-Whitney rank-sum test was utilized to compare the pathological scores. The level of significance was determined by the p-value. The summary results of the statistics were presented in terms of means SEM (n = 4).

Results

EtOH Concentrations in Urine and Body Weight

Throughout the duration of this study, all mice gained approximately 0.5 g/week, without variations in their food and treatment groups. In the EtOH treated mice, urine alcohol concentration ranged from 0 to 500 mg/dl [11]. The PRKCε ASO and the control group of the mice receiving EtOH did not show any significant differences in their mean urine alcohol levels of 284±78 mg/dl and 217±44 mg/dl, respectively. The mice in the control group had a liver weight percentage of 5.9±0.5 and in the EtOH-fed mice 9.7±0.3, with the latter group of mice having a PRKCε knockdown. It can thus be concluded that PRKCε silencing had no influence on the rise of liver weight.

Chronic EtOH Impact on Histologic and Plasma Liver Damage Markers

Different histological characteristics are visible in the micrographs presented in figure 1, including the pathology shown by H&E, steatosis shown with oil red O staining, and neutrophil infiltration shown with CAE staining. The details of the staining quantification and plasma transaminase results are given in table 1 and figure 2 (a-c) together with the described above histochemical properties shown in figure 1. The use of PRKCε ASO in controlling animal with access to diet had neither adverse nor beneficial effect on liver damage or plasma transaminases production. The four-week consumption of EtOH diet increased the total plasma concentration of transaminases (ALT and AST) while it also led to increased lipogenesis, inflammation and cellular death. The accumulation of fat in the liver due to the consumption of EtOH was partially prevented by the PRKCε ASO. It was evident that the administration of PRKCε ASO prevented the increasing accumulation of macro-vesicular fat, but it did not have any impact on accumulation of micro-vesicular fat. Though there was some protective effect on lipid accumulation, PRKCε ASO treatment did not significantly decrease the increase of inflammatory markers in liver and transaminases level caused by EtOH. In real sense, PRKCε ASO treatment caused quite a high increase in neutrophils level induced by EtOH consumption. Hence, it can be concluded that the usage of PRKCε ASO did not prohibit rising level of transaminases due to EtOH consumption.

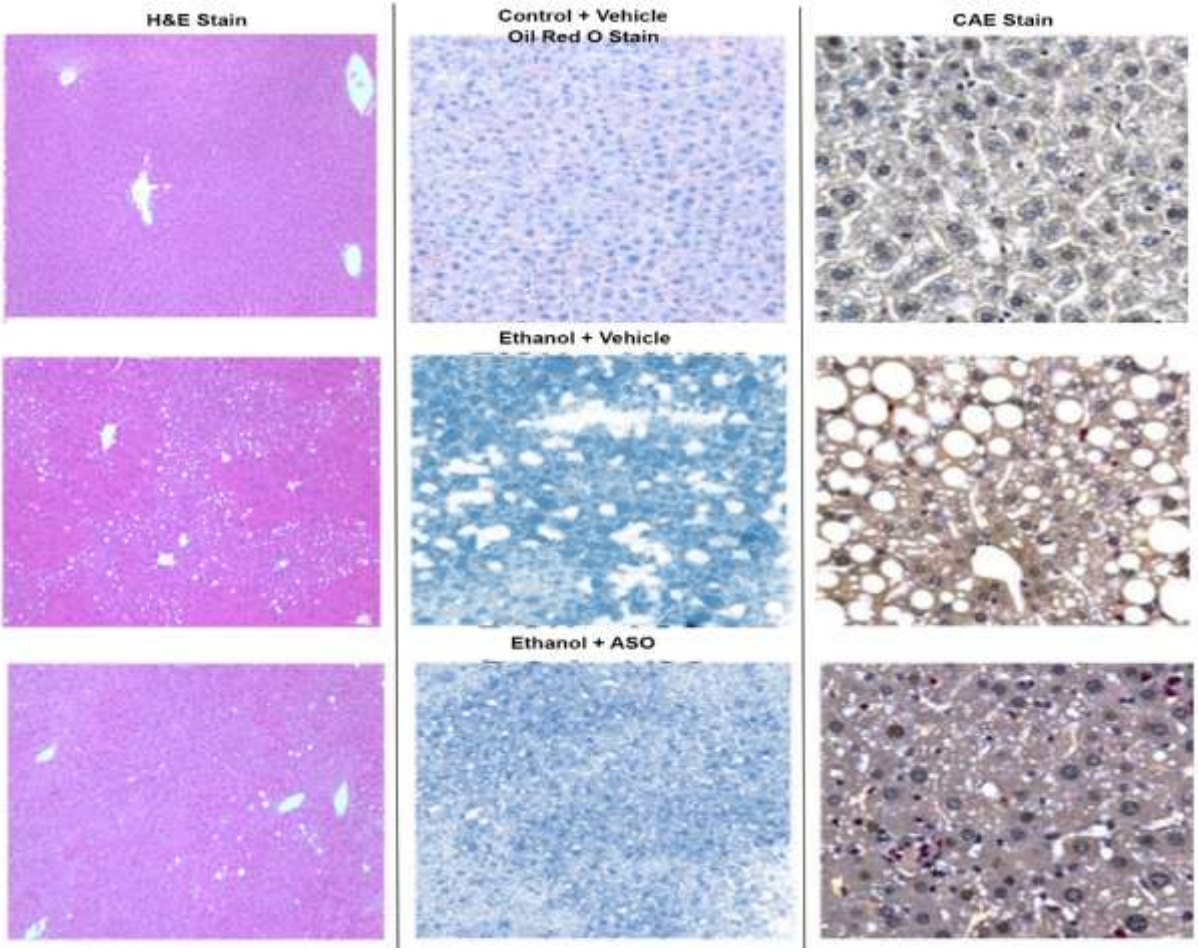
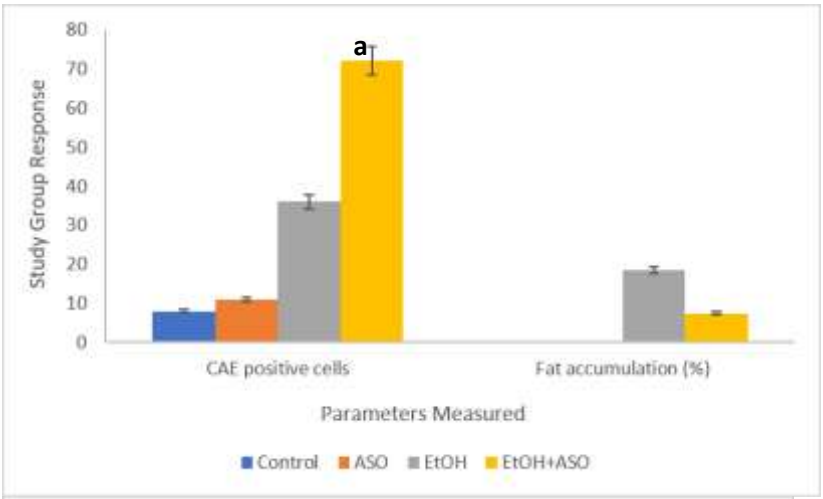
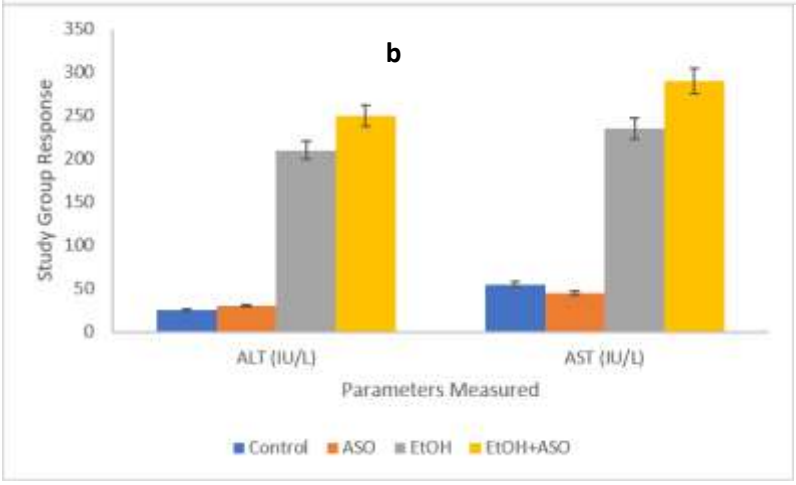


Figure 1: Photomicrographs of Different Stained Histology

Table 1: Staining Quantification and Plasma Transaminase Results

Parameters	Control	ASO	EtOH	EtOH+ASO
CAE positive cells	8	11	36	72
Fat accumulation (%)	0.2	0.2	18.5	7.5
ALT (IU/L)	25	30	210	250
AST (IU/L)	55	45	235	290
Inflammation score	0.3	0.3	1.65	1.95
Microvesicular steatosis	0	0	3	3
Macrovesicular steatosis	0	0	2.9	0.8
Necrosis score	0.3	0.3	1.65	1.95





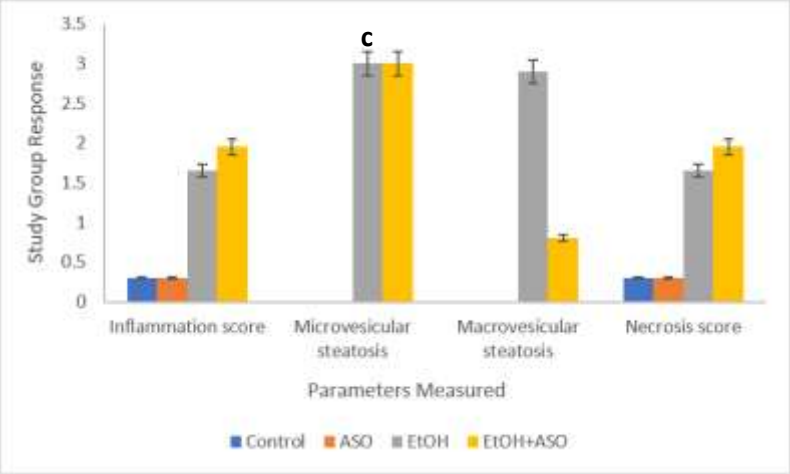


Figure 2: Damage of Liver Quantitation in terms of Histologic and Plasma Indices

EtOH's Impact on Hepatic DAG and PRKCε Activation

DAG can be considered one of the main activators of PRKCε [12] and thus its level in a liver due to long period of time consumption of EtOH was estimated. The DAG level appeared to be elevated in the liver of animals being fed with standard food and under the influence. In this regard, it is interesting to note that chronic alcohol intake remarkably increased DAG 36:4 in the liver from 129±26 pmoles/mg wet weight to 346±66 pmoles/mg wet weight. In the same manner, DAG 36:3 was raised from 72±4 pmoles/mg wet weight to 289±63 pmoles/mg wet weight with the use of alcohol. It is evident that EtOH enhances the process of translocation of PRKCε from cytosol to membrane, which signifies the enhancement of PRKCε activation at the level of protein and mRNA. In particular, it was proved that alcohol treatment provoked an increase in PRKCε translocation process. Thus, one could evaluate the effect of chronic EtOH intake over the PRKCε activation index. Chronic alcohol treatment resulted in the increase of membrane: cytosol ratio of PRKCε protein. Overall protein and mRNA levels remained almost unaltered with the help of EtOH treatment which proves the reliability of the findings presented by other scientists regarding PRKCε in NAFLD study [13].PRKCε ASO intervention decreased PRKCε protein and mRNA by 5 times almost. However, PRKCε mRNA levels appeared to be higher in alcohol-related cases comparing to control ones. Color imaging method was used to conduct the quantitative analysis of the lipids contained in the liver extracts obtained from the organ after lifting of the mice. The results obtained are represented in form of fold of control and represent the mean SEM (n = 4–6).

Effect of PRKCε Knockdown on EtOH-Induced Hepatic Lipid Changes

Histological analysis suggests that the PRKCε ASO lowered the effect of ethanol on liver fat accumulation. However, this staining method did not reveal what effects the ASO has on specific lipid pools increased by ethanol exposure. To resolve this issue, biochemical tests of hepatic triglycerides, fatty acids, cholesterol, and phospholipids were conducted (Figure 3). Chronic ethanol administration resulted in an increase in all of these lipids in mice liver. There was only an increase in phospholipids and cholesterol after administering the ASO to controls but not an increase in cholesterol caused by ethanol administration. The ASO had a significant impact on lowering triglycerides, free fatty acids and phospholipids accumulation by about 50%. The ASO was also proved to have a significant impact on several lipid subtypes as well.

Additions of EtOH significantly promoted alterations of each type of fatty acids analysed relative to the consequences arising from consumption of control diet, which is in accordance with results obtained for the total FFA measurements (Figure 3a). Moreover, the use of ASO greatly diminished the effects provoked by EtOH for the great part of these types. Thus, by way of example, the ASO relating to PRKCε diminished by approximately 50% increase of palmitic acid (16:0) accompanying addition of EtOH. This indicates the fact that the influence of ASO was consistent with the previous results for the FFA pool.

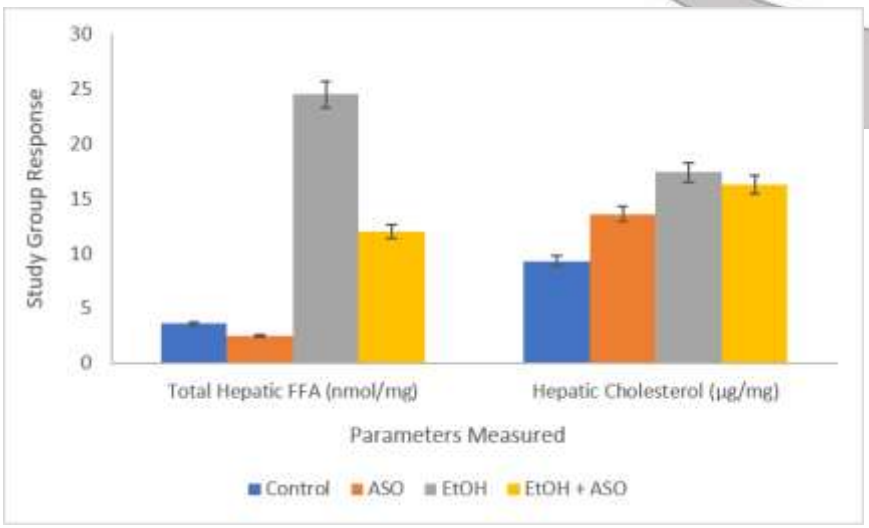


Figure 3: EtOH's Impact on Important Genes

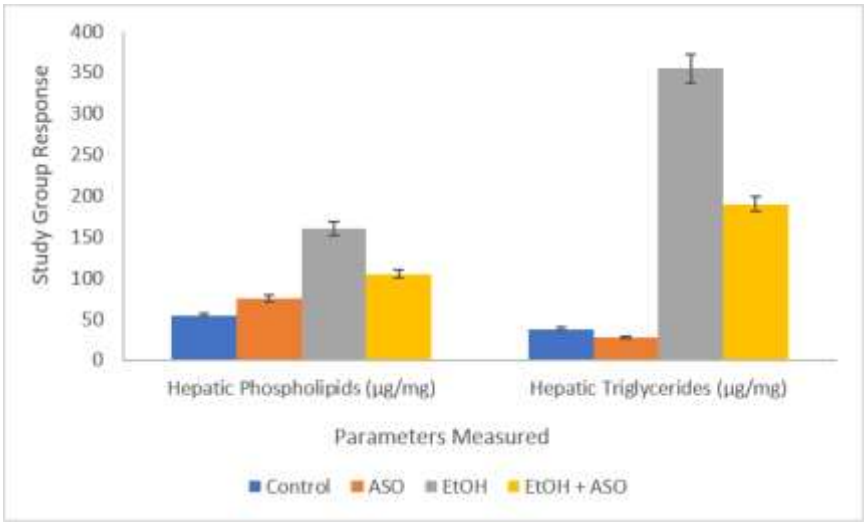


Figure 4: EtOH's Impact on Important Genes

The essence of changes is summarised in Figure 4 that reflects actions of EtOH with and without the influence from ASO targeted at PRKCε on all important markers of the processes which were found to be very important during the alcohol-induced liver injury. For example, fatty acid synthase (FAS) is one of the main gene of lipogenesis provoked by alcohol consumption [14]. The reduction of quantity of PRKCε leads to the lowering of TNFa production which is one of the most critical proinflammatory factors that are likely to take place in regards to experimental ALD [15]. Thus, the effect of PRKCε ASO as well as chronic EtOH on the gene expression of these markers was determined. EtOH resulted in the upregulation of the expression of all three genes several times, as expected. The PRKCε ASO, in contrast, completely inhibited the increase in the levels of expression of FAS and TNFa induced using EtOH, while this ASO did not have a substantial effect on the increase in the level of PAI-1 expression induced by EtOH under the same conditions (Figure 4).

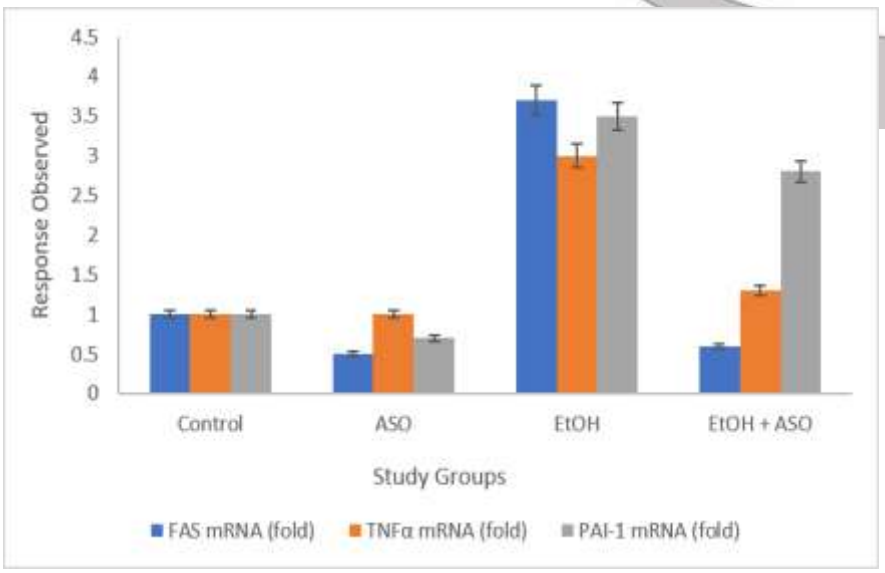


Figure 5: Effect of EtOH, with and without PRKCε ASO, on key indicators.

Chronic EtOH's Impact on Hepatic Fibrin Deposition

Previous studies indicated that hepatic inflammation is associated with the fact that PAI-1 inhibits the breakdown of fibrin [16,17]. Because of the fact that PRKCε ASO does not reduce the levels of PAI-1, therefore the effect of chronic EtOH on the liver and fibrin deposition was investigated. Confocal photomicrographs that show immunofluorescent detection of fibrin deposition were obtained and are presented in Figure 5. Long-term enteral administration of EtOH increased the deposition of fibrin in the liver. In accordance with the results of PAI-1 expression studies, PRKCε ASO therapy had no effect on the increase in the levels of fibrin deposition.

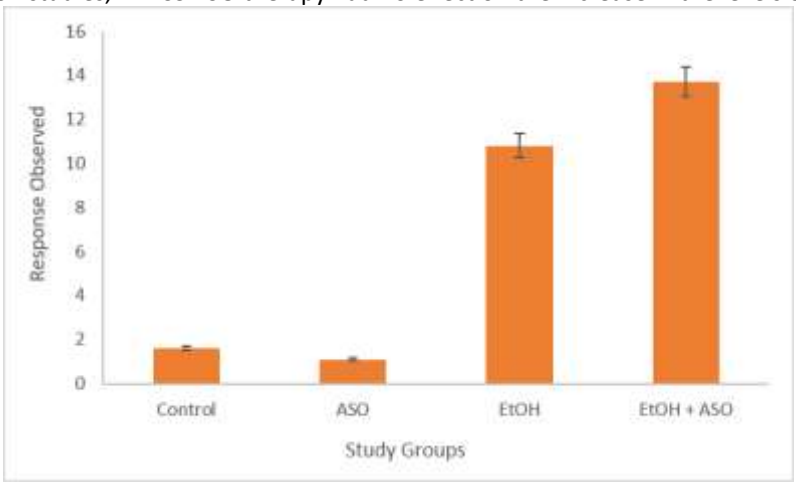


Figure 6: Fibrin Deposition Trend in the Presence of Ethanol

Discussion

Quite a few studies determined that lipid accumulation within liver is essential for the development of ALD. Hence, animal models which can reproduce early stage of ALD in humans are useful tools to investigate some mechanisms of action of Ethanol (EtOH) on liver. The mechanisms of ethanol-induced liver injury seem to be the same both in case of acute and chronic action. For example, the acute EtOH induced steatosis in rat liver can be prevented by substances which were found to act against the chronic ethanol-induced liver damage. Therefore, acute alcohol consumption may also mimic initial effects of EtOH on chronic liver injury. Previous studies suggested that the knock-down of PRKCε reduced the steatosis caused by the single bolus dose of EtOH in mice, which indicates some possible importance of this protein for the early stages of ALD [18]. It is important to understand that acute alcohol exposure models may not present all chronic alcohol effects on liver, although they are valuable for discovering new mechanisms and/or searching for new medications for ALD. Despite the

fact that the acute bolus model in the study of ethanol-induced changes in liver shows development of hepatic steatosis, this model completely lacks more severe diseases like inflammation and necrosis which are probably not connected with steatosis. Positive effects of the acute model are not sufficient for the further application in the field of health of human beings and development of alcohol-related liver disease.It was proven that, like in the acute experiment, silencing PRKCε brings about a process of hepatic steatosis related to periodical exposure to EtOH (Figure 1). Such state of accelerates both negative and positive reactions in lipid metabolism upon EtOH exposure. For example, alcohol influences at the same moment fatty acid beta-oxidation in the body inhibiting this process and activating the activity of fatty acid synthase. In this situation, ASO-treated mice which were subjected to EtOH consumption demonstrated disappearance of reactions related to the increase of enzyme level (Figure 4). Inhibition of the lipid pool accumulation occurs because of ASO influence on the inflammatory process in the liver. Therefore, ASO toxicity demonstrates that PRKCε has indirect effects in the process of ethanol-induced steatosis.Evidence for a direct role of PRKCε in FAS expression is lacking, although PRKCε has been shown to mediate TNFa expression and TNFa has previously been reported to increase levels of FAS in mouse liver. Thus, PRKCε knockdown might lead to reduced FAS production indirectly by inhibiting TNFa production (Figure 4). Interestingly, PRKCε knockdown led to an increase of cholesterol and phospholipids in the liver of those animals that received only control diet, but this increase was nevertheless lower than that observed on EtOH diet (Figure 2). Although the precise role of PRKCε in hepatic lipid metabolism remains incompletely understood, previous studies suggest that protein kinase C isoforms, including PRKCε, participate in phospholipid transport and cholesterol efflux [19]. Therefore, the alterations observed in hepatic lipid pools following PRKCε knockdown may reflect disturbances in these processes. In the present study, PRKCε knockdown partially attenuated macrovesicular steatosis, whereas microvesicular steatosis appeared to remain largely unaffected (Figure 1). Microvesicular steatosis is generally associated with impaired mitochondrial fatty-acid oxidation and may indicate mitochondrial dysfunction, suggesting that this component of ethanol-induced hepatic injury may occur independently of PRKCε signaling. The accumulation of hepatic protein and lipid following chronic ethanol (EtOH) exposure may contribute to hepatocellular hypertrophy and increased liver weight. Since PRKCε antisense oligonucleotide (ASO) treatment did not prevent the EtOH-induced increase in liver size, PRKCε inhibition alone may not be sufficient to completely prevent the metabolic and structural alterations responsible for hepatomegaly.

The classical “two-hit” hypothesis proposes that hepatic steatosis represents an initial sensitizing event that increases susceptibility to subsequent inflammatory and hepatocellular injury. Accordingly, inhibition of steatosis would be expected to reduce the severity of inflammation and necrosis. Because PRKCε inhibition reduced EtOH-induced steatosis, it was initially reasonable to hypothesize that it would also attenuate downstream inflammatory and necrotic changes. Moreover, previous studies have implicated PRKCε in EtOH-induced apoptosis and macrophage activation. However, the present findings were unexpected, as PRKCε ASO treatment reduced both hepatic steatosis and TNF-α levels but failed to prevent the development of inflammation and necrosis (Figure 1). These findings indicate that suppression of steatosis does not necessarily translate into protection against subsequent hepatic injury and suggest that inflammatory and necrotic pathways may be activated independently of PRKCε-mediated lipid accumulation.

This interpretation is consistent with previous observations involving diacylglycerol acyltransferase 2 (DGAT2), an enzyme involved in triglyceride synthesis. Studies have shown that reducing DGAT2 activity decreased hepatic steatosis in the methionine–choline-deficient model; however, despite the reduction in lipid accumulation, liver injury associated with inflammation was increased [20]. Thus, hepatic lipid accumulation may not simply represent a pathogenic mediator of liver injury but may also function as a protective mechanism by temporarily sequestering potentially lipotoxic fatty acids into relatively inert triglyceride stores. Consequently, the reduction of steatosis following PRKCε inhibition may not be sufficient to prevent inflammatory and necrotic injury and may explain why attenuation of lipid accumulation and TNF-α expression did not correspond to complete histological protection in the present study.[20]. According to the results obtained in this study, the high concentration of toxic FFAs can occur in the liver because of the inhibition of triglyceride synthesis due to the absence of DGAT2 expression. In this regard, no increase in the concentration of FFAs was found in the case of PRKCε ASO-treated animals. Furthermore, changes in the amount of this lipid pool were mainly downward. A more detailed analysis of some species of FFA proved that ASO reduced the amount of hepatotoxic FFAs, which appear because of EtOH administration. Therefore, even though there might have been a case of different approaches being used to avoid fatty liver and severe liver injury in animals in the previous study, this study cannot be explained by greater quantities of toxic FFAs.Increased hepatic cholesterol might also be another way inflammation can be maintained in our experiments. Indeed, it was shown that animal models deficient in LDLR and consuming high-fat and high-cholesterol diet, moreover, producing apolipoprotein E2, showed heavy

inflammation of the liver tissue due to the same reason that also influenced the hepatotoxic effect in Alms1 mutant (foz/foz) mice. Mitochondrial cholesterol may have a negative impact on liver tissues [21]. The failure of PRKCε ASO treatment to suppress the ethanol (EtOH)-induced increase in hepatic cholesterol (Figure 2) may provide another possible explanation for the persistence of hepatic inflammation despite the reduction in steatosis. Although the increase in cholesterol observed in the present study was less than two-fold, and substantially higher cholesterol concentrations have generally been required to induce marked hepatic injury in previous experimental studies, a contribution of cholesterol-mediated lipotoxicity cannot be completely excluded. Thus, cholesterol accumulation may represent an additional pathway through which EtOH promotes hepatic inflammation independently of the reduction in overall steatosis.

An important alternative mechanism may involve plasminogen activator inhibitor-1 (PAI-1), which has been implicated in nutritional and alcohol-induced hepatic inflammation. PAI-1 is a major inhibitor of fibrinolysis and promotes the persistence of fibrin matrices within the extracellular environment, thereby potentially contributing to inflammatory responses, tissue remodeling, and progression toward fibrosis. Previous studies have demonstrated that prolonged EtOH exposure increases PAI-1 expression and that PAI-1 contributes to the sensitization of the liver to subsequent inflammatory challenges, including lipopolysaccharide (LPS)-induced injury [22]. The present findings further suggest that the EtOH-induced increase in PAI-1 expression may occur through mechanisms that are largely independent of PRKCε. Therefore, PRKCε inhibition may reduce lipid accumulation without adequately suppressing PAI-1-dependent inflammatory and fibrogenic pathways.The reduction in steatosis and lipid accumulation observed following PAI-1 knockdown further supports the possibility that PAI-1 participates in both steatogenic and non-steatogenic responses to chronic EtOH exposure. Importantly, previous studies have shown that genetic deletion of PAI-1 protects animals against inflammation and necrosis induced by prolonged enteral alcohol exposure, with effects that extend beyond the reduction of hepatic steatosis [22]. Collectively, these observations suggest that PAI-1 may mediate the hepatic effects of EtOH predominantly through inflammatory mechanisms and, to a lesser extent, through its effects on hepatic lipid accumulation. This distinction may help explain why suppression of PRKCε-mediated steatosis did not completely prevent hepatic inflammation and necrosis in the present study.Neutrophil recruitment represents another important component of alcohol-induced hepatic inflammation. In the present study, PAI-1 administration approximately doubled the number of neutrophils accumulated in the liver following EtOH exposure (Figure 1). Excessive neutrophil recruitment and activation are recognized contributors to alcohol-associated liver injury, as activated neutrophils can release reactive oxygen species, proteolytic enzymes, and inflammatory mediators that promote hepatocellular damage. Consistent with this concept, previous investigations have demonstrated that inhibition of intercellular adhesion molecule-1 (ICAM-1), an important mediator of leukocyte adhesion and hepatic recruitment, significantly attenuates the inflammatory response during experimental enteral alcohol exposure [23].

Interestingly, despite the apparent increase in hepatic neutrophil numbers following PRKCε ASO treatment during EtOH exposure, this did not result in a corresponding increase in biochemical indicators of hepatocellular injury, such as serum transaminase levels. One possible explanation is that the total number of hepatic neutrophils does not necessarily reflect the number of functionally activated or tissue-damaging neutrophils. Thus, PRKCε ASO treatment may have altered neutrophil accumulation without substantially increasing their inflammatory activation or hepatotoxic activity. Overall, these findings indicate that PRKCε-dependent regulation of steatosis is mechanistically distinct from the pathways controlling PAI-1 expression, neutrophil activation, and hepatic inflammation. The persistence of inflammation and necrosis despite reduced steatosis therefore supports the concept that alcohol-induced liver injury involves multiple parallel pathways rather than a simple sequential relationship between steatosis and hepatocellular damage.

Conclusion

In conclusion, the results of the study provide additional evidence in support of the PRKCε's role in steatosis caused by EtOH. In addition, the study showed that PRKCε knockdown results in no decrease in inflammation, which is likely because it is unable to inhibit EtOH-induced PAI-1. This means that the idea that lipid accumulation is the only cause of EtOH-induced inflammation is put into doubt, as it shows that some additional mechanisms which accompany steatosis could become important. Therefore, it’s necessary to have in mind that It’s possible that even if those methods would succeed in reducing the steatosis caused by EtOH, they may not help in preventing the liver from being damaged.

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References

1. Li Y, Jiang Y, Hu Z, Yang S, Li L, Xiong C, Gao Y, Sun W, Zhang Y. Protein Kinase C Family: Structures, biological functions, diseases, and pharmaceutical interventions. MedComm. 2025 Nov;6(11):e70474.
2. Peng J, Zhang Y, Wang J, Zhang H, Schett G. Role of Chimeric Antigen Receptor–Expressing Cell Therapy in Immune-Mediated Kidney Diseases: A Review. American Journal of Kidney Diseases. 2025 Jun 6.
3. Al Dailaty A, Ghanem A, Abou Daher G, Chaaban T, Chatila R. Metabolic dysfunction-associated steatotic liver disease: an emerging comorbidity in COPD. Therapeutic Advances in Chronic Disease. 2025 Sep;16:20406223251378868.
4. Cai H, Zhu C, Zheng X, Lian Y, Tang E, Chen Y, Cao W, Sun Y, Lv Y. Knockout of IGFBP3 improves alcohol-induced liver injury via Akt/GSK3β and TMEM219/caspase 8 pathways. Frontiers in Physiology. 2026 May 15;17:1775461.
5. Jiang Y, Jiang K, Sun P, Liu Y, Nie H. Oroxylin A ameliorates non-alcoholic fatty liver disease by modulating oxidative stress and ferroptosis through the Nrf2 pathway. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2025 Jun 1;1870(5):159628.
6. Gandapur AJ, Wyatt TA. Alcohol use disorders and pneumonia: susceptibility and severity. Expert Review of Respiratory Medicine. 2026 Feb 1;20(2):159-71.
7. Banerjee D, Wei Z, Luyendyk JP. Role of the plasminogen activation system in liver injury and repair: knowns and known unknowns. Arteriosclerosis, Thrombosis, and Vascular Biology. 2026 Jan;46(1):27-36.
8. Skrabana R, Castven M, Tomková M, Sedlák E, Babulic P, Jakubcova M, Moskalets T, Leksa V. Rather a versatile multi-tool than a sword: an integral role of the plasminogen system in health and disease. Frontiers in Immunology. 2026 Jul 3;17:1878106.
9. Chen J, Ou G, Gu W, Shi J, Lyu R, Wu X, Wang J, Liu C. Role in preventing alcoholic liver disease progression: A comparative study of whole-component finger citron essential oil and its major component D-limonene. Nutrients. 2025 Apr 3;17(7):1255.
10. Lal-Shahsavar S, Zolbanin NM, Jafari A, Ghasemnejad-Berenji M. Metformin alleviates doxorubicin-induced hepatic damage by modulating oxidative stress: a molecular, biochemical, and histopathological approach in a rat model. Naunyn-Schmiedeberg's Archives of Pharmacology. 2025 Jul;398(7):9099-107.
11. McKim SE, Gäbele E, Isayama F, Lambert JC, Tucker LM, Wheeler MD, Connor HD, Mason RP, Doll MA, Hein DW, Arteel GE. Inducible nitric oxide synthase is required in alcohol-induced liver injury: studies with knockout mice. Gastroenterology. 2003 Dec 1;125(6):1834-44.
12. Shambhavi S, Mondal S, Chakraborty A, Shukla N, Panda BK, Kumar S, Kinatukara P, Pal B, Kamat SS, Sankaranarayanan R. Emergence of Dip2-mediated specific DAG-based PKC signalling axis in eukaryotes. Elife. 2025 May 6;14:RP104011.
13. Ali Z, Khan I, Waheed U, Chaudhary M, Lin Y, Younas S, Li J. Polysorbate 80 in Diabetes and Non-Alcoholic Fatty Liver Disease: Decoding Mechanisms of Hepatic Insulin Resistance and Its Cellular, Molecular, and Epigenetic Targets. Food Reviews International. 2026 Feb 17;42(2):514-39.
14. Huda N, Kusumanchi P, Jiang Y, Gao H, Thoudam T, Zeng G, Skill NJ, Sun Z, Liangpunsakul S, Ma J, Yang Z. Silencing FAF2 mitigates alcohol-induced hepatic steatosis by modulating lipolysis and PCSK9 pathway. Hepatology Communications. 2025 Mar 1;9(3):e0641.
15. Li Y, Kuang H, Fan G, Yang X. Alpha ketoglutaric acid attenuates LPS induced inflammatory response by inhibiting the PKCε/MAPK/P65 signaling pathway and inhibit oxidative stress in kupffer cells. Inflammation. 2025 Jun 27:1-1.
16. Li B, Fan H, Wu H, Zhang Y, Ding N, Liu P, Wang Q, Cao M, Meng Z, Feng X, Zhuo X. Targeting FXR in hepatocytes: a promising approach to enhance fibrinolysis and reduce deep vein thrombosis risk. Blood. 2025 Nov 13;146(20):2464-78.
17. Badran M, Gozal D. PAI-1: a major player in the vascular dysfunction in obstructive sleep apnea?. International Journal of Molecular Sciences. 2022 May 15;23(10):5516.
18. Xiao S, Lin R, Duan R, Li Z, Tang D, Liu X, Liu Y, Zhao M. Recombinant humanized IgG1 maintain liver triglyceride homeostasis through Arylacetamide deacetylase in ApoE−/− mice. International Immunopharmacology. 2022 Jul 1;108:108741.

19. Juhl AD, Wüstner D. Pathways and mechanisms of cellular cholesterol efflux—insight from imaging. Frontiers in Cell and Developmental Biology. 2022 Mar 1;10:834408.
20. Chauhan V, Malviya N. Design, Development and Ex-vivo Evaluation of Nanosponges Loaded Topical Gel for Rejuvenation of Skin. International Journal of Drug Delivery Technology. 2023;13(4):1277-1282.
21. Zhu W, Ma J, Zhang T, Zhu M, Duan Y, Yang X, Chen Y. Reversed role of CD36 deficiency in high-fat diet or methionine/choline-deficient diet-induced hepatic steatosis and steatohepatitis. Frontiers in pharmacology. 2025 Mar 5; 16:1522177.
22. Jyotishi A, Chauhan V, Jain SK, Vengurlekar S. Uncovering Protein Kinase Cε as a Central Regulator of Acute Hepatic Steatosis due to Ethanol. Int J Drug Deliv Technol. 2026;16(71s): 127-134. DOI: 10.25258/ijddt.16.71s.15
23. Rauchbach E, Zeigerman H, Abu-Halaka D, Tirosh O. Cholesterol induces oxidative stress, mitochondrial damage and death in hepatic stellate cells to mitigate liver fibrosis in mice model of NASH. Antioxidants. 2022 Mar 11;11(3):536.