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Hepatoprotective Effects of Aqueous Extract of *Perilla fructescens* against Alcohol-Induced Liver Injury in Mice

Ara Jo [1,†], Songiy Han [2,†], Sungchul Lim [3,†] and Chulyung Choi [1,4,*]

[1] Department of Integrative Biological Sciences & BK21 FOUR Educational Research Group for Age-Associated Disorder Control Technology, Chosun University, Gwangju 61452, Republic of Korea; joara9153@naver.com
[2] Division of Premedical Science, College of Medicine, Chosun University, Gwangju 61452, Republic of Korea; sihan@chosun.ac.kr
[3] Department of Pathology, College of Medicine, Chosun University, Gwangju 61452, Republic of Korea; sclim@chosun.ac.kr
[4] Institute of Well-Aging Medicare & Chosun University G-LAMP Project Group, Chosun University, Gwangju 61452, Republic of Korea
* Correspondence: blockstar@chosun.ac.kr
† These authors contributed equally to this work.

Abstract: Excessive alcohol intake leads to significant physiological complications, particularly alcoholic liver diseases (ALD). The extent of liver damage caused by ethanol correlates with increased oxidative stress and accumulation of lipids in the hepatic tissue. In this study, we investigated the defense properties of the aqueous extract of *Perilla frutescens* Briton Var. *acuta* Kudo (PF) on hepatic injury in chronically ethanol-treated mice. The mice were orally administered the water extract from PF for 4 weeks with ethanol treatment (3 g/kg. P.O.). The level of malondialdehyde (MDA) in the liver tissues was determined. A substantial increase in MDA generation was detected in the livers of mice subjected to ethanol exposure, whereas the administration of PF markedly reduced MDA levels in hepatic tissues. Additionally, histological analysis of the liver tissue was performed. Histopathological investigation revealed a significant reduction in hepatocellular necrosis in the PF-treated group. This study demonstrated that the aqueous extract of *Perilla frutescens* Briton Var. *acuta* Kudo (PF) attenuated chronic ethanol-induced liver injury by augmenting the antioxidant capacity of mice. These results can be utilized for the development of high-value-added products using PF.

Keywords: *Perilla frutescens* Briton Var; ethanol-induced liver injury; antioxidative capacity

Citation: Jo, A.; Han, S.; Lim, S.; Choi, C. Hepatoprotective Effects of Aqueous Extract of *Perilla fructescens* against Alcohol-Induced Liver Injury in Mice. *Processes* **2024**, *12*, 1404. https://doi.org/10.3390/pr12071404

Academic Editors: Francesca Raganati and Dariusz Dziki

Received: 17 May 2024
Revised: 14 June 2024
Accepted: 4 July 2024
Published: 5 July 2024

1. Introduction

Abusive alcohol consumption poses a significant global health concern and substantially contributes to morbidity and mortality [1]. Moreover, persistent and excessive alcohol consumption can lead to alcoholic liver disease (ALD), a condition that has been the primary cause of cirrhosis and liver-related fatalities globally for decades, representing approximately 4% of mortality globally [2–4]. ALD encompasses a spectrum of liver pathologies histologically, commencing with steatosis (fatty liver) and progressing to alcoholic steatohepatitis (ASH). In severe cases, it may progress to fibrosis, cirrhosis, or eventually hepatocellular carcinoma [5]. Hence, the management of ALD in its early stages, before the emergence of ASH, is important to prevent its progression. Despite the complex nature of ALD pathogenesis, growing evidence suggests that ethanol-triggered oxidative stress contributes significantly to its progression [6–8].

The liver predominantly metabolizes approximately 90% of alcohol into carbon dioxide and water through oxidation, with only a minor portion undergoing enzymatic breakdown through the action of alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) [9]. Elevated alcohol intake can increase the activity of cytochrome P450 enzymes in

the liver, resulting in excessive generation of reactive oxygen species (ROS) and subsequent lipid peroxidation [10]. Acetaldehyde, an intermediary in alcohol metabolism, is converted to superoxide- and oxygen-free radicals by xanthine oxidase, thereby compromising the structural integrity and functionality of hepatocytes. This sequence accelerates oxidative stress-induced liver damage [11,12].

Chronic alcohol consumption has been implicated in multiple medical conditions, including cancer, diabetes, cardiovascular disease, and liver and pancreatic disorders. In severe alcoholic hepatitis, prednisolone is indicated as the first-line treatment even with the possibility of various complications. Early liver transplantation can be another option for highly selected patients with a null response to prednisolone. Most importantly, abstinence is the mainstay of long-term care, but relapse is frequent among patients. At present, abstinence remains the most important treatment for ALD [13,14].

The natural product *Perilla fructescens* Briton Var. *acuta* Kudo (PF) is a medicinal plant belonging to the phylum Magnoliophyta, class Magnoliopsida, and family Asteraceae [15]. *Perilla frutescens*, an annual herb of the Labiatae family, is a perennial herbaceous plant found in Republic of Korea and China. PF leaves, stems, and seeds can be used as medicine and edible food [16]. Because of the abundant nutrients and bioactive components in this plant, PF has been studied extensively in medicine, food, health care, and chemical fields with great prospects for development. Several studies have reported the presence of rich polyphenolic compounds, which exhibit high antioxidant capacity, in Perilla leaves. Perilla essential oil (PEO), extracted from PF leaves, is a complex mixture of volatile components, constituting approximately 150 to 200 different compounds, exhibiting high antioxidant, anticancer, anti-inflammatory, insecticidal, and antimicrobial activities [17–19]. Modern medical research has revealed that different plant parts of PF possess enormous amounts of bioactive secondary metabolites, including terpenoids, flavonoids, alkaloids, steroids, quinines, and phenolic compounds, which exhibit a wide range of biological activities and have immense potential applications as pharmaceuticals, nutraceuticals, agrochemicals, biopesticides, flavours, fragrances, colours, and food additives. Different biological activities, including anti-allergic, anti-depressant, hypolipidemic, hepatoprotective, neuroprotective, anti-inflammatory, anticancer, antioxidant, and antimicrobial activities, were reported in PF [7,20]. Moreover, PF has protective effects against t-BHP-induced hepatotoxicity in rat liver through the modulated activity and expression of CYP1A1/2 and HO-1, as well as the activation of Nrf2, suggesting that PF extract could be beneficial in treating several diseases associated with oxidative stress through its enhanced antioxidant mechanisms and may also have therapeutic applications in liver diseases involving oxidative damage [21].

Consequently, PF holds significant promise for the treatment of ALD owing to its hepatoprotective properties. This study aimed to explore the hepatoprotective effects against alcohol-induced liver damage, formulate nutraceuticals, and isolate sourced from natural reservoirs to relieve hangovers. Ultimately, this research aimed to provide insights into potential strategies for addressing alcohol-induced liver damage and hangover relieving using natural nutraceuticals.

2. Materials and Methods

2.1. Botanical Specimens and Extract Preparation

PF leaves were provided by the Jeonnam Institute of National Resources Research (Jangheung-Gun Jeollanam-Do, Republic of Korea). Air-dried branches and leaves were cut into small pieces, and chopped PF leaves (3 kg) were extracted with 30 L of distilled water at 100 °C for 3 h. The extracted solution was filtered through a 400-mesh filter, followed by concentration using a vacuum rotary evaporator. After filtration, the residue was subjected to the same process twice with an equivalent volume of distilled water for extraction, filtration, and vacuum concentration. The concentrated aqueous extract was freeze-dried using a freeze dryer, resulting in 390 g (yield 13.01%) of PF leaf aqueous extract.

2.2. Preparation of Fractions

Fractions were prepared from aqueous extracts using an organic solvent for polarity of PF leaves. To prepare a nonpolar solvent-soluble fraction, 250 g of PF leaves in aqueous water extract were completely dissolved in 1 L of distilled water, and a fractionation filter was used. Following this, 1 L of hexane was used to separate the water and hexane layers, and the process was repeated three times. Each fraction was obtained by sequentially adding chloroform ($CHCl_3$), ethyl acetate (EtOAc), and butanol using the same process. Each fraction was filtered and concentrated using vacuum filtration and then freeze-dried to completely remove the solvent. Preparative HPLC was performed using a YL HPLC system equipped with a UV detector. A UV detector was used at 254 and 324 nm, and an Agilent Eclipse XDB-C18 4.6 × 250 mm was used for HPLC (Agilent Technologies, Inc., Santa Clara, CA, USA). Gradient elution was performed using methanol (MeOH) and 0.1% formic acid in water. The mobile phase was as follows: 0–15 (15%), 15–25 (25–100%), 25–32 (100%), 32–36 (35%), 35–42 min (35%).

2.3. Animal Study

Male ICR mice (4 weeks old, weighing 22–24 g) were obtained from Samtako Bio Korea (Osan, Republic of Korea). The mice were housed under standardized conditions at a consistent room temperature of 20–23 °C with a humidity level of 50 ± 5%, and ad libitum access to water and standard rodent chow was provided under a 12 h light–dark cycle (lights on at 08:00 a.m.). A period of 7 days was allotted for animal acclimatization prior to commencing the experiments, and mice were group-housed according to their respective experimental groups. In advance, 36 mice were randomly assigned to six groups as acute Ethanol-induced mice (n = 6/group): normal group (oral saline administration), control group (Oral ethanol and saline administration), PF 10 mg/kg group (ethanol and PF 10 mg/kg oral administration), PF 50 mg/kg (ethanol and PF 50 mg/kg oral administration), PF 100 mg/kg (ethanol and PF 100 mg/kg oral administration), and PF 200 mg/kg (ethanol and PF 200 mg/kg oral administration). The acute-induced mouse group was administered the extract orally once daily for 7 days. On the 7th day, 30 min after the final PF treatment, all groups except the normal group were administered a single oral dose of ethanol.

Additionally, the chronic ethanol-induced group was randomly divided into six groups, each consisting of 36 mice (n = 6/group): normal group (oral saline administration), control group (oral ethanol and saline administration), PF 10 mg/kg group (ethanol and PF 10 mg/kg oral administration), PF 50 mg/kg (ethanol and PF 50 mg/kg oral administration), PF 100 mg/kg (ethanol and PF 100 mg/kg oral administration), and PF 200 mg/kg (ethanol and PF 200 mg/kg oral administration). All mouse groups were orally administered daily for 4 weeks, and except for the control group, all groups were administered ethanol daily, beginning 30 min after oral administration of PF for 4 weeks. On the final day of each experiment, fasting and water deprivation were implemented after administration of the final sample and inductor. After an 18 h interval, ether anesthesia was administered and laparotomy was performed to collect blood from the abdominal aorta. The serum was separated by centrifugation at 1500 rpm for 10 min. Liver tissue samples were excised and immediately fixed in 4% paraformaldehyde. The remaining liver tissue was collected and preserved at −70 °C for further experimental protocols.

2.4. Measurement of Blood Alcohol Concentration

To measure the plasma alcohol concentration, the protein was denatured by treatment with 10% trichloroacetic acid, twice the amount of blood collected per hour, centrifuged at 1000× g for 15 min, and the supernatant was collected. Blood alcohol levels were determined using an ethanol assay kit (Biovision Inc., Mountain View, CA, USA). The approach entailed the use of commercial assay kits in accordance with manufacturer's guidelines, with absorbance at 570 nm.

2.5. Biochemical Parameters in the Serum

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in the serum were determined using a spectrophotometric diagnostic kit (ASAN medical center, Seoul, Republic of Korea). For ALT measurement, 0.2 mL of serum separated from blood were mixed with 1 mL of substrate solution (Alanine, α-Ketoglutarate mixture) in a test tube and incubated at 37 °C for 30 min. Subsequently, 1 mL of 2,4-Dinitrophenylhydrazine was added, gently mixed, and allowed to react at room temperature for 20 min. Then, 10 mL of a solution containing Sodium hydroxide diluted precisely 10 times with purified water were added, mixed, and left at room temperature for 10 min. Absorbance was measured at 505 nm using a spectrophotometer.

For AST measurement, 0.2 mL of serum separated from blood were mixed with 1 mL of substrate solution (aspartate, α-Ketoglutarate mixture) in a test tube and incubated at 37 °C for 30 min. Subsequently, 1 mL of 2,4-Dinitrophenylhydrazine was added, gently mixed, and allowed to react at room temperature for 20 min. Then, 10 mL of a solution containing sodium hydroxide diluted precisely 10 times with purified water were added, mixed, and left at room temperature for 10 min. Absorbance was measured at 505 nm using a spectrophotometer.

2.6. ADH and ALDH Activity Assays

Mice were sacrificed at 1 h of alcohol administration and the blood was collected. The serum was rapidly stored at −70 °C until analysis, and serum was mixed with each assay buffer of ADH Activity Colorimetric Assay (Biovision Inc., Mountain View, CA, USA).

ALDH activity was measured as follows: mice were sacrificed at 4 h of alcohol administration and the liver was dissected out. The isolated liver was rapidly frozen in liquid nitrogen, stored in −70 °C until analysis, and washed with phosphate-buffered saline (PBS) before analysis. Liver homogenate was mixed with assay buffer of ALDH activity kit (Biovision Inc., Mountain View, CA, USA). After mixing, the procedure was performed according to manufacturer's instructions and absorbance was measured at 450 nm.

2.7. Assessment of Lipid Peroxidation Levels in the Liver

The concentration of lipid peroxidation in liver tissue was determined using the thiobarbituric acid (TBA) method with commercial detection kits following manufacturer's instructions (Biovision Inc., Mountain View, CA, USA). Liver tissue was homogenized by adding 1 mL of PBS to a homogenizer glass tube followed by 195 µL of 72% Trichloroacetic acid (TCA; final conc. 10%) and centrifugation at 12,000 rpm at 4 °C for 15 min. The supernatant was used as fresh EP, transferred to a tube, and centrifuged again. The supernatant (0.6 mL) was transferred to a new EP tube, 272 µL with 0.8% TBA (final conc. 0.25%) were added, and the mixture was boiled at 95 °C. When the color of the control changed to pink, the ice reacted. Subsequently, 100 µL of supernatant were transferred to a 96-well plate, and the amount of malondialdehyde (MDA) produced was measured. MDA was used as the reference standard, and the results were expressed as nmol MDA equivalents per milligram of protein (mg protein).

2.8. Histopathological Examination

Liver tissue specimens were fixed in 4% (*v*/*v*) formalin and embedded in paraffin. The paraffin-embedded samples were sectioned into 4–5 µm-thick sections, and each section was stained with hematoxylin and eosin for histopathological evaluation. Fixed samples were dehydrated in 70%, 80%, 90%, 95%, and 100% ethanol, cleared with xylene, and infiltrated with paraffin wax before embedding in paraffin blocks. Tissue sections of 4 µm thickness were prepared and mounted on poly-L-lysine-coated slides. For H&E staining, tissue sections were deparaffinized in xylene, followed by dehydration through a series of 100%, 95%, 80%, and 70% ethanol, and then rinsed in distilled water. Sections were immersed in hematoxylin solution for 7 min, rinsed again in distilled water, differentiated in 1% HCl alcohol, rinsed in distilled water, briefly dipped in 0.5% ammonia water to stain nuclei

with phosphate groups in blue, rinsed in distilled water, counterstained with 1% Eosin to stain cytoplasm or connective tissue in pink, dehydrated again in 80%, 95%, and 100% ethanol, cleared in xylene, and mounted for microscopic examination and photography of tissue sections.

2.9. Statistical Analysis

All data are presented as mean ± standard deviation (SD). One-way analysis of variance was performed, followed by post-hoc analysis using GraphPad Prism for Windows (GraphPad Software version 5, San Diego, CA, USA). Group differences were evaluated using the Duncan's multiple-range test. Statistical significance was set at $p < 0.05$.

3. Results

3.1. Organic Solvent Fractions Obtained from PF and HPLC Analysis

The PF extract was obtained using solvents such as hexane, $CHCl_3$, EtOAc, BuOH, and water (Figure 1). The yield of the hot water extract from PF leaves was 13%. Using 3 kg of PF leaves, we performed two rounds of hot water extraction and freeze-drying, resulting in a final yield of 390 g of PF hot water extract. From 250 g of this PF hot water extract, we fractionated it according to solvent polarity in the following order: hexane, $CHCl_3$, EtOAc, and butanol. The yields obtained from each fraction were as follows: hexane 0.015 g (yield 0.006%), $CHCl_3$ 0.57 g (yield 0.23%), EtOAc 3.62 g (yield 1.45%), butanol 54.74 g (yield 21.9%), and water 150.14 g (yield 60.1%).

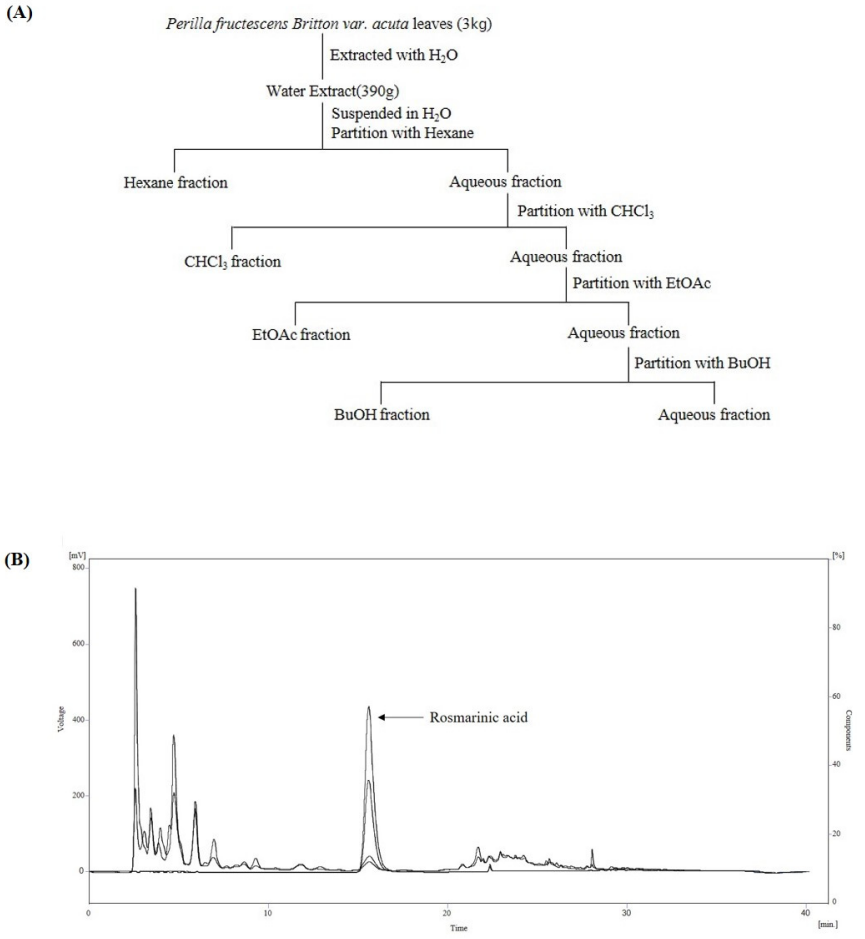

Figure 1. Fractionated process of aqueous leaves extracts from *Perilla fructescens* and high-performance liquid chromatography analysis (**A**) fractionation procedures for PF are outlined through a schematic diagram. PF was dissolved in water and partitioned with hexane, $CHCl_3$, EtOAc, and BuOH. (**B**) High-performance liquid chromatography chromatograms of Rosmarinic acid of *PF* extract.

And according to previous results, the components of Perilla extract include anthocyanins, essential oils, vitamins, minerals, and rosmarinic acid [5,6,8–10]. When the rosmarinic acid content of perilla extract decreased, the immune-boosting effect decreased. Rosmarinic acid plays a pivotal role in immune responses. Therefore, rosmarinic acid was confirmed to be an indicator component of PF leaf extracts through HPLC analysis.

3.2. Effect of PF Extract on Blood Alcohol Concentration

PF leaf extract was administered orally 30 min before ethanol administration. Blood was collected every 30 min, 1 h, 2 h, and 4 h to measure the blood alcohol concentration, as shown in Figure 2. Similarly, the effect of reducing blood alcohol concentration was confirmed and compared 2 h after ethanol administration. Specifically, as a result of comparing the blood alcohol concentration 2 h after administering ethanol, in the experimental group that was orally administered, 200 mg/kg of PF leaf hot water extract and ethanol were administered. Blood alcohol concentration decreased by 194.41 mM in the experimental group compared to that in the control group (257.56 mM). As a result of measuring the blood alcohol concentration when the PF extract was treated at 50 ($p < 0.05$), 100 ($p < 0.05$), and 200 mg/kg ($p < 0.005$), it was confirmed that the blood alcohol concentration decreased in a concentration-dependent manner. Thus, the group that administered PF showed lowering blood alcohol concentration, which is expected to be the result of promoting alcohol decomposition enzymes.

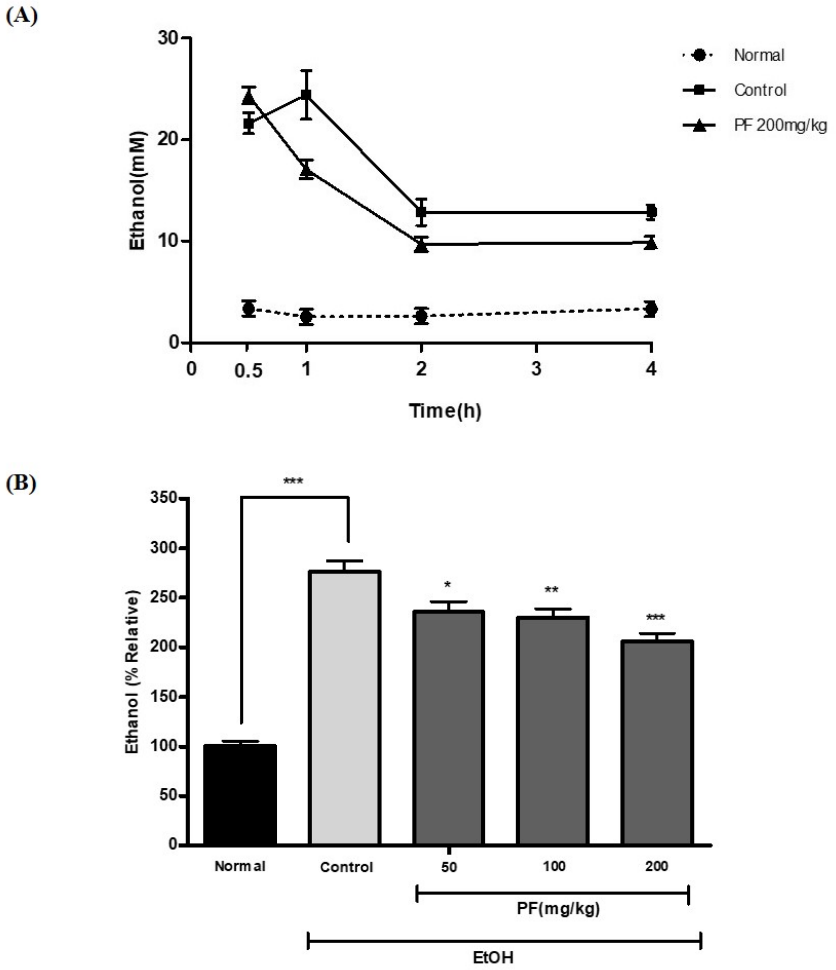

Figure 2. Effects of PF extract on blood alcohol concentration after administration of ethanol in mice. (**A**) Blood alcohol concentrations at 0.5, 1, 2, and 4 h after ethanol administration (**B**) Measurement of blood alcohol concentration using PF extract at 50, 100, and 200 mg/kg, respectively. Data are expressed as mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ compared to that of the EtOH group.

3.3. Effect of PF Extract on ADH and ALDH Activities

To assess the impact of *PF* extract on alcohol metabolism enzymes relative to alcohol intake, ADH and ALDH enzyme activities in the liver tissue were measured (Figure 3). ADH activity was higher in the PF extract-treated group than in the EtOH group ($p < 0.05$). In particular, we confirmed a dose-dependent significant increase in ADH activity with PF extract treatments at 50, 100, and 200 mg/kg ($p < 0.005$) concentrations. The effect of alcohol-metabolizing enzyme activity is also related to the improvement of hangovers, and it is affected by the absorption and excretion of alcohol, the amount of enzymes involved in the alcohol decomposition metabolic process, and the activity of the enzyme. Following alcohol consumption, ADH in the liver metabolizes alcohol, decreasing blood alcohol concentration and generating acetaldehyde, a principal metabolite that contributes to the symptoms of hangovers. Natural materials that promote ALDH enzymatic activity are known to relieve hangovers. Both ADH and ALDH activities were significantly higher in the PF extract-treated group than in the EtOH group. Particularly, ALDH activity was significantly higher than ADH activity, confirming that it had a greater effect on hangover relief. Thus, PF extract can help relieve hangovers.

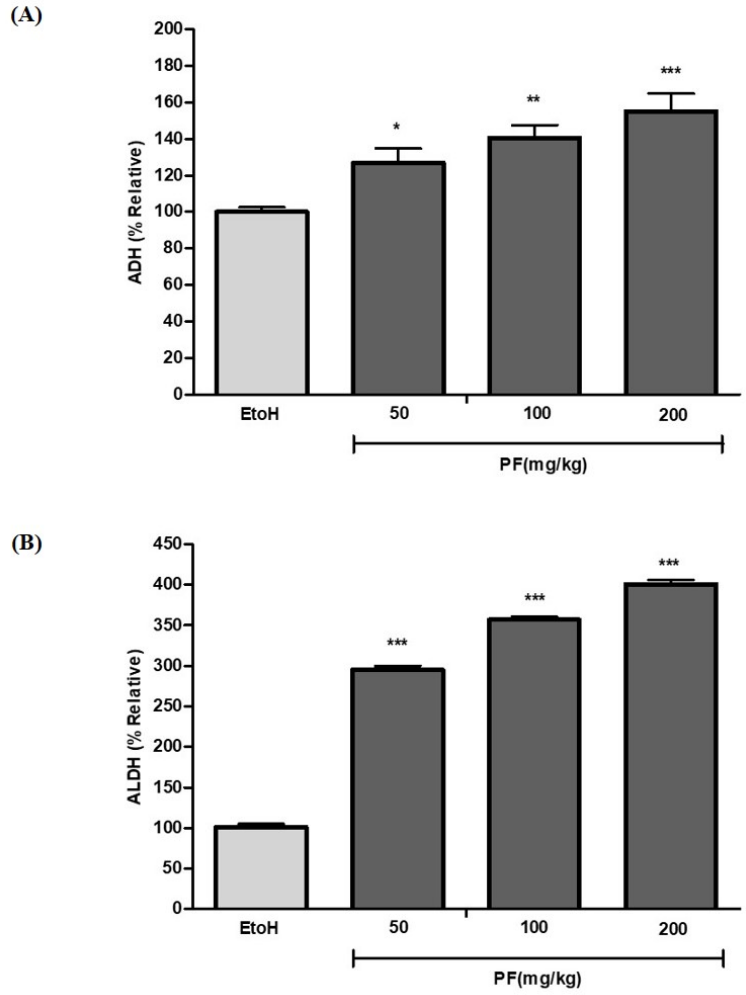

Figure 3. Effects of water extract from PF on alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) activity in ethanol-induced mice. (**A**) PF extract at doses of 50, 100, and 200 mg/kg was administered 30 min before oral ethanol administration, and ADH activity in the serum was measured 1 h after ethanol administration. (**B**) PF hot water extract at doses of 50, 100, and 200 mg/kg was administered orally at each concentration 30 min before ethanol administration, and ALDH activity in the liver tissue was measured 4 h after ethanol administration. Data are expressed as mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$ compared to that of the EtOH group.

3.4. Impact of PF Extract on AST and ALT Levels in Acute Ethanol-Induced Mice

To evaluate the hepatoprotective effects of PF extract in an acute ethanol-induced mouse model, the concentrations of ALT and AST, which are pivotal blood biochemical markers of liver function, were assessed (Figure 4A,B). ALT and AST are enzymes commonly employed in the diagnosis of liver disease because their release into the bloodstream from impaired hepatocytes and liver tissue reflects the progression of liver toxicity. Consequently, the activity of these enzymes in the bloodstream increases, which is a marker of liver damage. Thus, the quantification of ALT and AST levels served as indicators for assessing the hepatoprotective effects of PF extract in the context of acute ethanol-induced liver injury in mice. The examination of serum AST and ALT levels revealed no significant difference following treatment with PF extract. This suggests that PF extract does not induce hepatotoxicity and may be safely used as a material without causing liver damage.

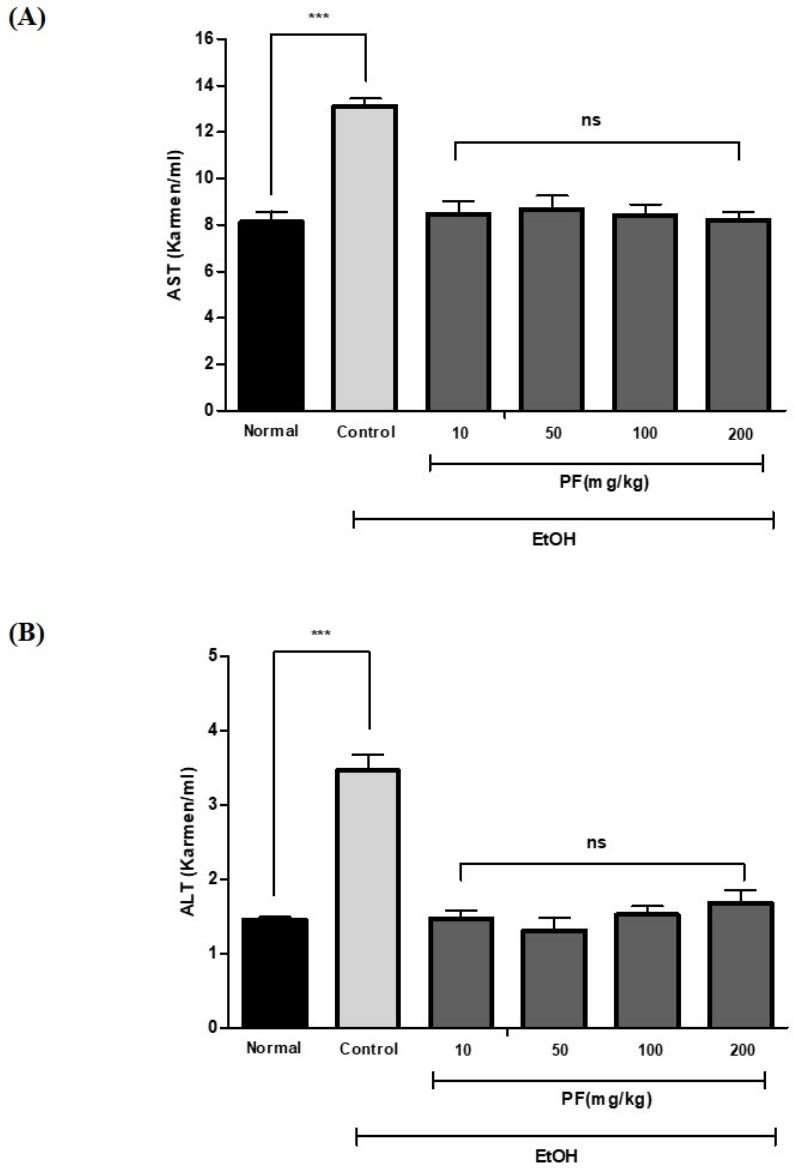

Figure 4. *Cont.*

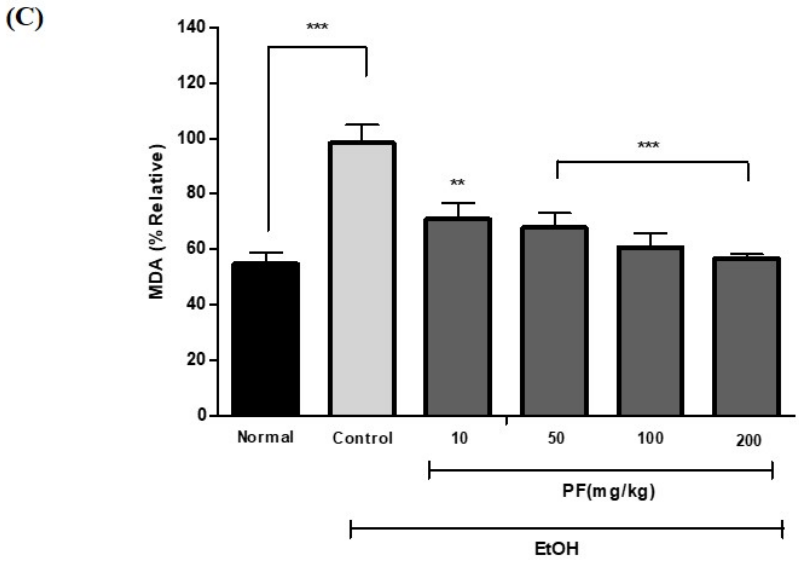

Figure 4. Effects of water extract from PF on ALT and AST activities and MDA in acute ethanol-induced mice. In the acute ethanol-induced mouse model, hepatotoxicity was assessed by administering PF water extract at doses of 10, 50, 100, and 200 mg/kg. Hepatotoxicity was evaluated by measuring the serum concentrations of (**A**) AST and (**B**) ALT. Serum AST and ALT levels were determined using a spectrophotometric diagnostic assay kit. (**C**) MDA levels were measured in liver tissue homogenates from the acute ethanol-induced mouse model to assess acute liver injury induced by PF water extract. Data are expressed as mean ± SD. ns; no significance ** $p < 0.01$, *** $p < 0.005$.

3.5. Effect of PF Extract on MDA Levels in Acute Ethanol-Induced Mice

Oxidative stress plays a pivotal role in the advancement of alcohol-induced liver damage and pathogenesis of ALD. To mitigate oxidative stress, various enzymatic and non-enzymatic defense mechanisms have evolved to counteract the effects of ROS generated during alcoholic liver injury. MDA is the primary byproduct of ROS-triggered lipid peroxidation. Furthermore, MDA levels can indirectly indicate the extent of cellular damage caused by free radical assaults in the organism. MDA levels were assessed in the liver tissues of mice subjected to acute ethanol-induced injury (Figure 4C). The results revealed a significant increase in lipid peroxidation in the EtOH group compared to that in the control group. However, treatment with PF extract at doses of 10 ($p < 0.01$), 50, 100, and 200 mg/kg ($p < 0.005$) in acute ethanol-induced mouse liver tissues showed a slight concentration-dependent decrease in MDA levels, suggesting the potential mitigation of ethanol-induced oxidative stress.

3.6. Effect of PF Extract on AST and ALT Levels in Chronic Ethanol-Induced Mice

As depicted in Figure 5, serum AST and ALT levels were significantly elevated in the Chronic EtOH exposure group compared with those in the normal group. As observed in the serum results of acute ethanol-induced mice, the administration of PF extract did not yield any significant differences in AST and ALT levels.

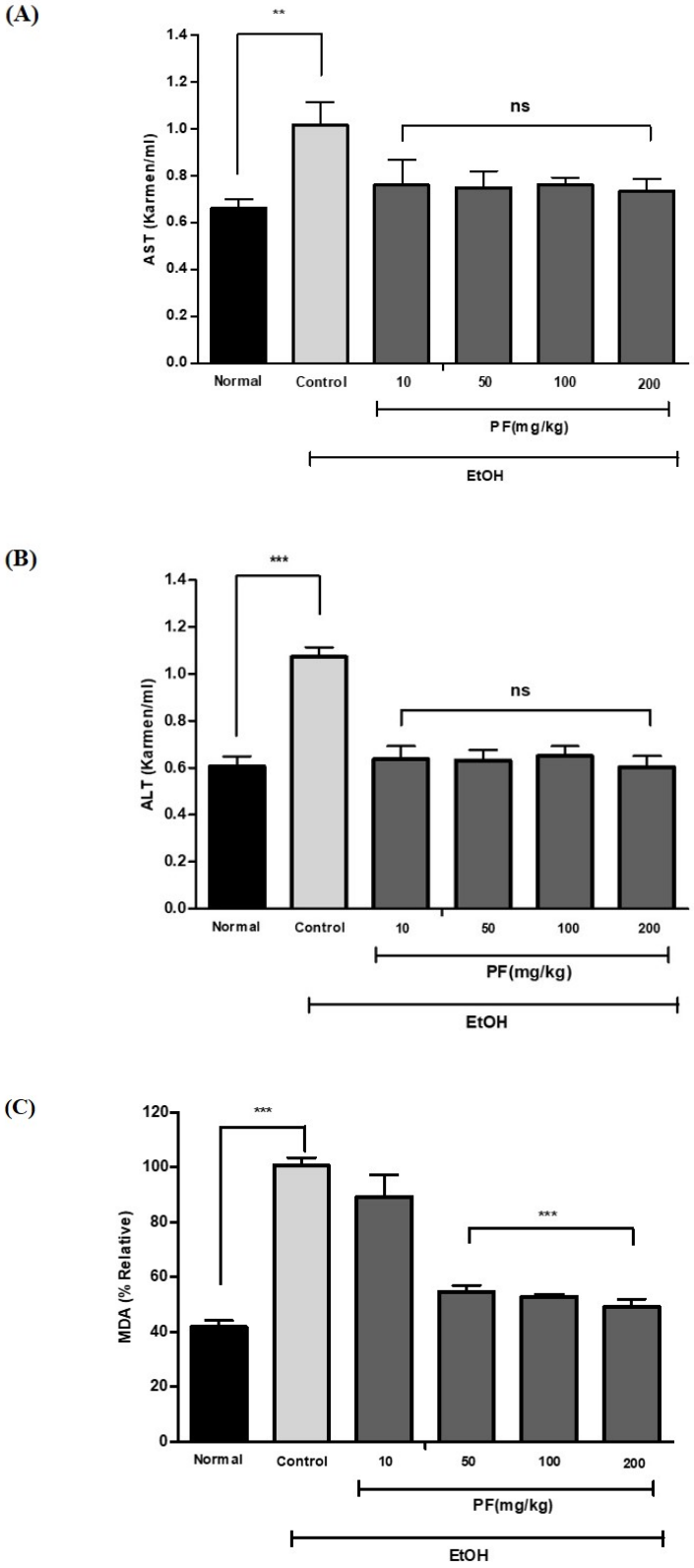

Figure 5. Effects of water extract from PF on ALT and AST activities and MDA in chronic ethanol-induced mice. In the chronic ethanol-induced mouse model, hepatotoxicity was assessed by administering PF water extract at doses of 10, 50, 100, and 200 mg/kg. Hepatotoxicity was evaluated by measuring the serum concentrations of (**A**) AST and (**B**) ALT. Serum AST and ALT levels were determined using a spectrophotometric diagnostic assay kit. (**C**) MDA levels were measured in liver tissue homogenates from the acute ethanol-induced mouse model to assess acute liver injury induced by PF water extract. Data are expressed as mean ± SD. ns; no significance, ** $p < 0.01$, *** $p < 0.005$.

3.7. Effect of PF Extract on MDA Levels in Chronic Ethanol-Induced Mice

The levels of lipid peroxidation in the liver tissues of mice exposed to chronic alcohol consumption are shown in Figure 4. Compared with the control group, the ethanol-treated group exhibited a substantial increase in lipid peroxidation levels. However, treatment with PF extract at doses of 10, 50, 100, and 200 mg/kg ($p < 0.005$) in chronic ethanol-induced mouse liver tissues showed a slight concentration-dependent decrease in MDA levels.

3.8. Effect of PF Extract on Pathological Analysis in Acute and Chronic Ethanol-Induced Mice

To assess histopathological changes in the liver tissues of mice with acute alcohol-induced liver injury following treatment with PF leaf extract, the liver tissues were harvested on the 3rd day after ethanol induction (Figure 6A). In the control group, abundant cell nuclei were observed surrounding the blood vessels. Conversely, in the ethanol group, a significant reduction in the number of cell nuclei around the blood vessels was evident owing to induced lipid peroxidation, indicating hepatocellular necrosis. In contrast, despite ethanol-induced liver injury, the group treated with the PF leaf extract exhibited a widespread distribution of cell nuclei around the blood vessels and throughout the tissue, indicating a concentration-dependent reduction in ethanol-induced liver injury. Thus, histopathological observations of liver tissues demonstrated that PF leaf extract mitigated acute liver injury by inhibiting ethanol-induced lipid peroxidation. Furthermore, the histopathological analysis of liver tissues from mice with chronic alcohol-induced liver injury treated with the PF leaf extract revealed a notable reduction in ethanol-induced liver damage, which is consistent with the observations in tissues with acute alcohol-induced liver injury (Figure 6B).

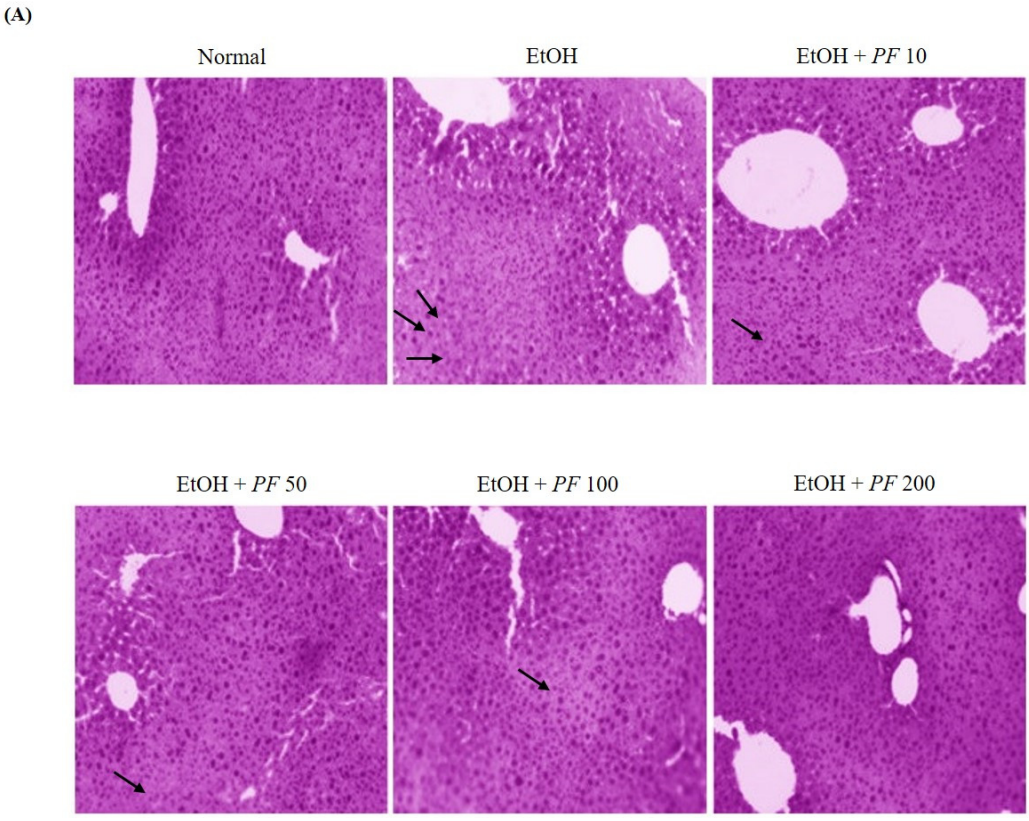

Figure 6. *Cont.*

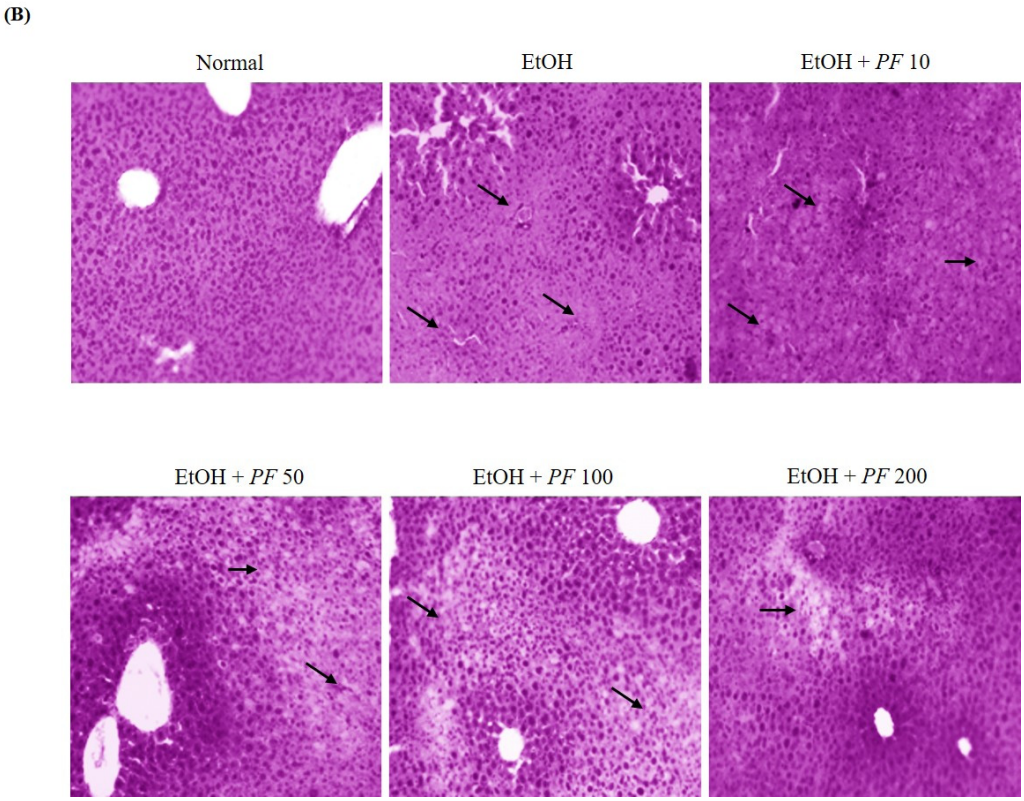

Figure 6. Effect of water extract from PF on alcohol-induced histopathological changes in acute and chronic liver tissue. (**A**) Acute alcohol-induced mice. (**B**) Chronic alcohol-induced mice. Hematoxylin and eosin staining. Original magnification, ×200.

4. Discussion

Alcohol misuse has become a significant global health challenge, imposing notable social and economic burdens [22,23]. In the human body, alcohol is primarily metabolized in the liver. Brief episodes of heavy alcohol consumption can inflict severe harm on the liver, elevating the risk of acute alcoholic liver disease. Additionally, prolonged alcohol consumption can lead to conditions such as fatty liver disease, liver cirrhosis, and hepatocellular carcinoma [24–26].

Upon consumption, alcohol elicits hangovers via metabolites such as acetaldehyde, with ALD and ALDH enzymes playing pivotal roles in alcohol metabolism. One hypothesis suggests that these enzymes can be stimulated through the pharmacological effects of extracts from natural sources. Figure 1 illustrates that treatment with *PF* extract effectively lowered blood ethanol levels in an ethanol-induced mouse model, indicating a probable enhancement of ethanol metabolism within the bloodstream. This phenomenon is likely attributable to the heightened activities of ADH and ALDH enzymes observed in the ethanol-induced mouse model.

Persistent alcohol consumption induces liver damage, leading to changes in the transport function and membrane permeability of hepatic cells. Consequently, enzymes are discharged from the cells into the bloodstream, resulting in increased levels of AST and ALT. Substantial elevations in ALT and AST levels signify severe impairment of the liver tissue membrane due to alcohol or drug intoxication. Therefore, we investigated the effect of PF extract on both acute and chronic alcohol-induced liver injury in mice. Ethanol consumption results in liver impairment, as indicated by increased levels of AST and ALT enzymes in the serum, which are indicative of liver damage. However, treatment with PF extract led to a reduction in these elevated enzyme levels, suggesting a protective effect against alcohol-induced liver injury. The normalization of AST and ALT levels implied hepatocyte regeneration and healing of liver tissue, further supporting the hepatoprotective

properties of PF extract. Additionally, the study observed histopathological changes in the liver associated with acute and chronic alcohol-induced damage. Treatment with PF extract also significantly improved these histopathological alterations, offering additional evidence of the hepatoprotective effects of PF extract against alcohol-induced liver injury in mice. Overall, PF extract exhibited protective effects against alcohol-induced liver injury, as demonstrated by biochemical markers and histopathological observations in the liver tissue.

Various factors and mechanisms contribute to the pathological progression of liver damage induced by alcohol consumption, with oxidative stress being the most prominent [27]. ROS, including hydroxyl radicals, superoxide radicals, and hydrogen peroxide, are produced during metabolism. Under normal circumstances, ROS is efficiently neutralized by the antioxidant defense system of the body. However, chronic alcohol exposure leads to an imbalance, resulting in the excessive accumulation of ROS and subsequent cellular damage. Of particular concern is lipid peroxidation in hepatocytes, which is considered the primary mechanism underlying chronic alcohol-induced liver injury [28]. MDA, a product of ROS-induced lipid peroxidation, accumulates in alcohol-damaged livers and serves as a reliable indicator of overall oxidative stress [29–34].

Alcohol consumption significantly increased lipid peroxidation levels, whereas treatment with PF extract normalized these levels. This demonstrates the significant hepatoprotective effect of PF extract, which is attributed to its ability to mitigate lipid peroxidation and subsequent oxidative stress in the liver caused by alcohol exposure.

In this study, PF extract demonstrated a protective effect against both acute and chronic alcohol-induced liver damage. Since PF extract contains numerous bioactive compounds, the hepatoprotective effect could stem from the collective action of multiple mechanisms. However, elucidating the specific mechanisms of action remains challenging. The antioxidant properties of PF and reduction in MDA levels in vivo suggest that PF may mitigate ethanol-induced oxidative stress, thereby exerting hepatoprotective effects.

To identify the active components contained in the water extract of PF, we obtained fractions using organic solvents. Through HPLC analysis, we confirmed the presence of rosmarinic acid in each extract and fraction. We propose that rosmarinic acid is the active compound with hepatoprotective effects in both acute and chronic ethanol-induced mouse models. This study confirms that rosmarinic acid is the substance responsible for hepatoprotective activity in ethanol-induced mouse models. Future research will aim to further verify the hepatoprotective efficacy of rosmarinic acid. Additionally, we plan to continue studying additional active substances in the fractions obtained using organic solvents from the PF water extract.

Through additional research, we will analyze the hepatoprotective effects of fractions obtained using organic solvents derived from PF aqueous extracts against ethanol-induced oxidative stress. Furthermore, since this study is a preclinical investigation using mice, the molecular mechanisms underlying the hepatoprotective effects of PF aqueous extract are not fully elucidated, so further research is planned. Based on these research findings, PF extracts will be proposed as a promising material for the development of health-functional foods that can alleviate the effects of excessive alcohol consumption by alleviating hangovers and protecting liver health.

Furthermore, PF water extract exhibits hepatoprotective effects against alcohol-induced liver damage without toxicity, making it safe for long-term consumption of its active constituents. This natural plant extract can be utilized effectively for the protection against both acute and chronic alcohol-induced liver injury, offering the advantage of cost-effectiveness due to the use of locally available plant resources. The industrial utilization of PF extract could potentially reduce import dependency and enhance export opportunities.

Author Contributions: Conceptualization, C.C.; formal analysis, A.J.; investigation, S.H. and S.L.; writing—original draft preparation, A.J.; writing—review and editing, A.J. and C.C.; project administration, C.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by Global-Learning & Academic research institution for Master's PhD students, and the Postdocs (LAMP) Program of the National Research Foundation of Korea (NRF) grant funded by the Ministry of Education (NO. RS-2023-00285353).

Institutional Review Board Statement: The animal study protocol was approved by the Institutional Review Board (or Ethics Committee) of Chosun University IACUC (Institutional Animal Care and Use Committee) (protocol code CIACU2023-S0032 and 8 November 2023).

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of Interest: The authors declare no conflict of interest.

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