Dear Editor and Reviewers,

We sincerely appreciate your insightful comments and constructive suggestions on our manuscript entitled "A new strain of *Pediococcus pentosaceus* alleviates ethanol-induced liver injury by modulating the gut microbiota and short-chain fatty acid metabolism" (Manuscript NO: 57791). The concerns of the reviewers and their suggestions have been carefully studied and addressed in the revised manuscript. Corrections in the paper and responses to the reviewers' comments are listed below. We also completed and formatted the submitted manuscript according to WJG guidelines for authors and the Editor's suggestions. We hope that our manuscript will be suitable for publication in World Journal of Gastroenterology and look forward your response.

Sincerely,

Lanjuan Li

State Key Laboratory for Diagnosis and Treatment of Infectious Disease, The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, China. E-mail: ljli@zju.edu.cn

Responses to the Editor's comments:

1. The "Approved Grant Application Form(s) or Funding Agency Copy of any Approval Document(s)" has been provided.

2. Editable figures in PowerPoint format has been uploaded.

3. The "Article Highlights" section has been added to the revised manuscript.

4. The superscript letters of *P* values have been revised.

5. All of the suggested editorial changes have been made.

Response to the reviewers:

Reviewer #1 (Reviewer's code: 03733090):

Reviewer comments to authors This paper mainly observed the regulatory effect of the probiotic *Pediococcus pentosaceus* on intestinal microflora and SCFA metabolism and found that *P. pentosaceus* could improve the intestinal barrier function of mice with alcoholic liver injury, reduce the levels of circulating endotoxin, proinflammatory cytokines and chemokines, and reduce alcoholic liver inflammation and steatosis. The results have important reference value in the research and treatment of alcoholic liver injury. However, there are still some shortcomings need clarification in this paper. M & M: 1. Please explain why the number of animals is different between the experimental groups, the control group (n = 8), the EtOH group (n = 10), and the *P. pentosaceus* group (n = 10). 2.The time point of collection of fresh feces needs to be explained clearly.

Results: 3. Many of the descriptions in the results belong to the background or discussion, and should be transferred to the background or discussion, respectively. Such as, “Gut-derived lipopolysaccharide translocated to the liver, which promoted immune cell activation via Toll-like receptors and the release of cytokines and chemokines to enhance liver inflammatory responses.“ “Overall, *P. pentosaceus* supplementation reduced the systemic levels of endotoxin and proinflammatory cytokines to alleviate the hepatic inflammatory response.“ “The intestinal barrier protects against bacterial translocation from the gastrointestinal tract to the liver, so we further investigated whether *P. pentosaceus* supplementation improved gut barrier function to decrease LPS levels.“ “Previous studies reported that ethanol-induced intestinal bacterial overgrowth contributed to bacterial translocation, so we evaluated the overall bacterial load with qPCR using 16S rRNA primer sets. “The antibacterial peptides Reg3ß and Reg3γ are secreted by epithelial cells to restrict bacterial overgrowth, and the gene expression of Reg3ß and Reg3γ was determined by qPCR”. Discussion: 4. “isocaloric maltose dextrin” needs to be mentioned in the discussion References: 5. Magazine abbreviations are not standardized. Such as, references 7, 15, 18, 20, 22, 23, 24, 29, 31, 36, 41, 42, 44, 46, 47 and 50 Figures legend: 6. The title of figure should not be a result of conclusion of the study, but just like Figure 7, it outlines the issues of the figure and explains the various signs in the figure. The rest figures (Figures 1-6, and Supplementary Figure 1) need to rewrite. (The titles in Figure 5 and

Figure 4 are identical?) 7. Scales =? in figures 1 and 3 8. Language description needs further improvement.

1. Please explain why the number of animals is different between the experimental groups, the control group (n = 8), the EtOH group (n = 10), and the *P. pentosaceus* group (n = 10).

Response: Thank you for the question. At the beginning of the experiment, the mice were divided into three groups, and the control group received a pair-fed control diet that without ethanol; therefore, no mice in the control group would have died during the experiment. However, the mice in EtOH group and *P. pentosaceus* group received a Lieber-DeCarli diet that contained 5% ethanol. In particular, the administration of one dose of ethanol (5 g/kg) by gavage on day 11 might have resulted in an increased mortality rate[1]. Therefore, more mice were included in the EtOH group and *P. pentosaceus* group at the beginning of experiment to ensure that the subsequent results were more reliable.

2. The time point of collection of fresh feces needs to be explained clearly.

Response: Thank you for the comment. The fresh cecal contents were collected after the mice were sacrificed on day 11 and immediately stored at -80°C until use. This information has now been added to the Materials and Methods section.

3. Many of the descriptions in the results belong to the background or discussion, and should be transferred to the background or discussion,

respectively. Such as, “Gut-derived lipopolysaccharide translocated to the liver, which promoted immune cell activation via Toll-like receptors and the release of cytokines and chemokines to enhance liver inflammatory responses.“ “Overall, P. pentosaceus supplementation reduced the systemic levels of endotoxin and proinflammatory cytokines to alleviate the hepatic inflammatory response.“ “The intestinal barrier protects against bacterial translocation from the gastrointestinal tract to the liver, so we further investigated whether P. pentosaceus supplementation improved gut barrier function to decrease LPS levels.“ “Previous studies reported that ethanol-induced intestinal bacterial overgrowth contributed to bacterial translocation, so we evaluated the overall bacterial load with qPCR using 16S rRNA primer sets. “The antibacterial peptides Reg3ß and Reg3γ are secreted by epithelial cells to restrict bacterial overgrowth, and the gene expression of Reg3ß and Reg3γ was determined by qPCR”.

Response: Thank you very much for the valuable comments and suggestions. Based on your suggestion, the inappropriate descriptions were deleted from the Results and transferred to the Introduction and Discussion sections.

4. “isocaloric maltose dextrin” needs to be mentioned in the discussion

Response: Thank you for the suggestion. In our study, the EtOH group and *P. pentosaceus* group were administered a single dose of ethanol (5 g/kg) by gavage on day 11, and control groups were administered the same volume of isocaloric maltose dextrin solution by gavage. “Isocaloric maltose dextrin” was

used as the control reagent for ethanol in the chronic and binge NIAAA model[1]. The necessary explanations were added to the Materials and Methods section.

5. Magazine abbreviations are not standardized. Such as, references 7, 15, 18, 20, 22, 23, 24, 29, 31, 36, 41, 42, 44, 46, 47 and 50.

Response: Thank you for the suggestion. We have revised the format of journal abbreviations in the references section as suggested.

6. The title of figure should not be a result of conclusion of the study, but just like Figure 7, it outlines the issues of the figure and explains the various signs in the figure. The rest figures (Figures 1-6, and Supplementary Figure 1) need to rewrite. (The titles in Figure 5 and Figure 4 are identical?)

Response: Thank you for the insightful suggestion. We have revised the figure legends (Figures 1-6 and Supplementary Figure 1) as suggested.

7. Scales =? in figures 1 and 3.

Response: Thank you for the kind reminder of this point. The phrases "Scale bar: 50μm" and "Scale bar: 100μm" were added to the Figure Legends section (Figures 1 and 3).

8. Language description needs further improvement.

Response: Thank you for the insightful comments. We have proofread the manuscript again to correct the grammatical and language errors, and the revised manuscript was further reviewed and polished by American Journal Experts.

Reviewer #2 (Reviewer's code: 01518946):

This manuscript describes the effect of a new strain of Pediococcus pentosaceus administration on the ethanol-induced liver injury. The authors found down-regulation of expression of inflammatory cytokines in the ethanol-induced liver injury of mouse models with administration of this bacterium. As a result, form correlation network model, the authors found the involvement of short chain fatty metabolism in the reduced live injury. Although detailed molecular mechanism remains unknown, this manuscript is hot topic in alcoholic liver injury and intestinal bacteria.

1. One more experiment should be performed before publication. The authors should investigate the effect of other strain of *Pediococcus pentosaceus* and or other bacteria on ethanol-induced liver injury.

Response:

Thank you very much for the insightful and constructive comments. Based on your suggestion, we also have been investigated the effects of other bacteria on ethanol-induced liver injury. The probiotics *Lactobacillus salivarius* LI01 (CGMCC 7045) and *Clostridium butyricum* MIYAIRI 588 were also selected to evaluate the protective effect on the chronic and binge NIAAA model. *Lactobacillus salivarius* LI01 (*L. salivarius*) was originally isolated from a healthy volunteer in our laboratory and *Clostridium butyricum* MIYAIRI 588 (*C. butyricum*) was isolated by Miyarisan Pharmaceutical Co., Ltd. As shown in the attached figure (Figure 1), the hepatic triglyceride levels and fat accumulation

in hepatocytes in the histological images were not significantly different between the probiotics group and EtOH group. Moreover, the supplementation of *Lactobacillus salivarius* LI01 or *Clostridium butyricum* MIYAIRI 588 did not significantly decrease the ALT and AST levels compared with the EtOH group. The preliminary results indicated that the supplementation of *Lactobacillus salivarius* LI01 or *Clostridium butyricum* MIYAIRI 588 did not exert a protective effect on ethanol-induced liver steatosis and injury. Therefore, we did not perform further studies using these two strains of probiotics: *Lactobacillus salivarius* LI01 and *Clostridium butyricum* MIYAIRI 588. In addition, recent studies have also reported the protective effects of different probiotics on ethanol-induced liver injury[2-4]. *Akkermansia muciniphila*, *Lactobacillus rhamnosus* GG, *Roseburia intestinalis* have proven to be effective at protecting against ethanol-induced hepatic steatosis and inflammation by regulating the gut microbiota and gut barrier function in murine ALD models, but further human clinical studies are needed to evaluate their safety and efficacy as treatments for ALD. Thus, not all of the probiotics exert a protective effect on ethanol-induced liver injury, and the cause of the preventive effects of an identified single microbial species and the associated molecular mechanisms require further study.

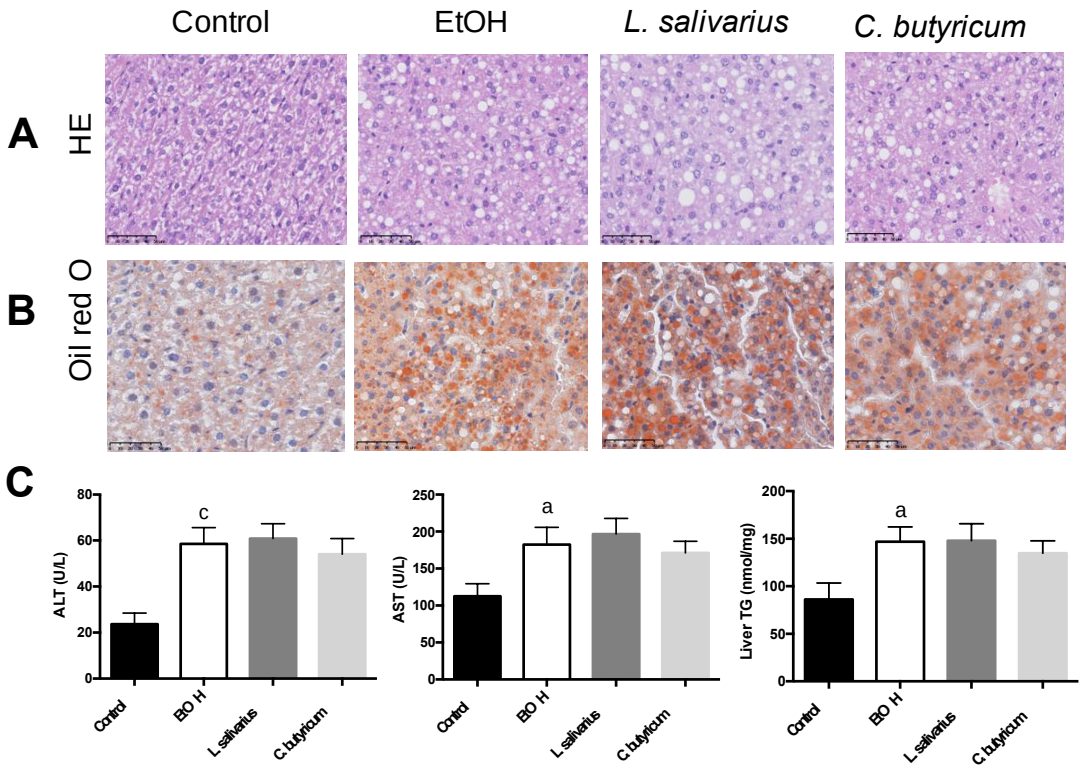

Figure 1. The hepatic histopathological examination and liver injury and steatosis parameters. (**A**) Representative histological images of the liver stained with HE. Scale bar: 50μm. (**B**) Representative images of oil red O-stained liver sections. Scale bar: 50μm. (**C**) Quantification of triglyceride in the liver and ALT and AST levels. All data are presented as means ± SEM. $^{a}P < 0.05$, $^{c}P < 0.001$, compared with the Control group.

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