

PEER-REVIEW REPORT

Name of journal: *World Journal of Gastroenterology*

Manuscript NO: 69541

Title: Dual therapy with zinc acetate and rifaximin prevents from ethanol-induced liver fibrosis by maintaining intestinal barrier integrity

Provenance and peer review: Unsolicited Manuscript; Externally peer reviewed

Peer-review model: Single blind

Reviewer's code: 03650105

Position: Peer Reviewer

Academic degree: MD

Professional title: Doctor

Reviewer's Country/Territory: China

Author's Country/Territory: Japan

Manuscript submission date: 2021-07-06

Reviewer chosen by: AI Technique

Reviewer accepted review: 2021-07-09 06:41

Reviewer performed review: 2021-07-17 14:00

Review time: 8 Days and 7 Hours

Scientific quality	<input type="checkbox"/> Grade A: Excellent <input type="checkbox"/> Grade B: Very good <input checked="" type="checkbox"/> Grade C: Good <input type="checkbox"/> Grade D: Fair <input type="checkbox"/> Grade E: Do not publish
Language quality	<input type="checkbox"/> Grade A: Priority publishing <input checked="" type="checkbox"/> Grade B: Minor language polishing <input type="checkbox"/> Grade C: A great deal of language polishing <input type="checkbox"/> Grade D: Rejection
Conclusion	<input type="checkbox"/> Accept (High priority) <input type="checkbox"/> Accept (General priority) <input type="checkbox"/> Minor revision <input checked="" type="checkbox"/> Major revision <input type="checkbox"/> Rejection
Re-review	<input checked="" type="checkbox"/> Yes <input type="checkbox"/> No

Peer-reviewer statements	Peer-Review: [<input checked="" type="checkbox"/>] Anonymous [<input type="checkbox"/>] Onymous Conflicts-of-Interest: [<input type="checkbox"/>] Yes [<input checked="" type="checkbox"/>] No
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SPECIFIC COMMENTS TO AUTHORS

Overview of the manuscript The aim of this manuscript is to investigate the preventive effect of combined zinc supplementation and rifaximin from ALD-related liver fibrosis induced by ethanol+CCl₄ in mice, which maintain intestinal barrier integrity and reduce hepatic LPS exposure, leading to Kupffer cell expansion and hepatic stellate cell via inhibiting the toll-like receptor 4 pathway. This is a well-written paper containing interesting idea which somewhat merit publication after several important issues to be addressed. Details 1. Abstract section needs to use a structural style, that is, four parts for Aim, Method, Results, and Conclusion, limited to 250 words. 2.As described, rifaximin inhibited toxin-induced apoptosis and deprivation of tight junction proteins (TJPs) in human intestinal cells through pregnane X receptor (PXR)-dependent inhibition of the TLR4/ MyD88/ NF- κ B pathway. Should the authors supplement PXR-related tests? 3. Most of the tests are based on PCR. However, due to the influence of post transcriptional regulation etc., mRNA does not necessarily affect the phenotype. This is also the core bug of this research. They should use multiple detection methods to explain the same phenotypic change, rather than explain different phenotypic changes by the same detection method, which is easy to produce a certain degree of arbitrary conclusion. Has the author considered how to explain this issue? 4. Figure 1. C57BL/6 mice were treated with ETOH + CCl₄ for 8 weeks; however, the author is requested to provide the reasons for choosing 8 weeks as the treatment time, for instance, previous literature, previous research of their own or time-dependent gradient experiments, etc. Serum/Hepatic concentration of oxidative stress (CAT, MDA, and SOD), liver function (ALB, ALP, and GGT) and blood lipid (TG ,TC, HDL-C, and LDL-C) as well as in vivo

dose of the drug (LPS and RFX) should be determined, meanwhile, the author should provide Oil Red O staining results in liver tissues and quantitative analysis. 5. Figure 2. What are the changes of metabolic enzymes related to alcohol, acetaldehyde and cytochrome (CYP2E1) in mice liver tissues when induced by Lieber -Decarli liquid diet and CCl₄? Could rescue work after the dual-intervention with zinc acetate and rifaximin? Such experiment should be supplemented. There are multiple biomarkers of macrophages, such as F4/80, CD68, and RAM11, etc. The reasons for selecting F4/80 positive cells in this study should be stated. In addition, the author should consider to distinguish M1 and M2 subtypes macrophage after treated with Lieber -Decarli liquid diet and CCl₄. 6. Figure 3. The changes of NF- κ B and IK- κ B are opposite. Should the authors consider using IK- κ B to verify the Western Blotting results. Besides, WB bands of p-p65 in E/RFX, p65 in E/Zn and E/RFX were quite different in only 2 samples. Better WB images should be provided. Experiment with western blotting and immunohistochemistry should be supplemented to evaluate protein changes of extracellular matrix accumulation in liver fibrosis of E/V group. 7. Figure 4. Western blotting for tight junction protein (such as Zo1 and Occludin) and immunofluorescence for Occludin protein should be added. High-magnification of HE staining and immunofluorescence images should also be provided. 8. Figure 5. The authors should provide changes in cell electrical resistance values with line chart during cell culturing. Concentration of EtOH and LPS need to be detected at lower compartment after 5% EtOH or 2 μ g/ml LPS added to culture medium of upper compartment in Transwell plates. In the same way, protein levels of tight junction should be detected. 9. Supplementary Figure 1. BUN should be detected together with serum creatinine for kidney function. 10. Besides, for the sake of data transparency, scatter chart or histogram should be used for the full text statistics.

RE-REVIEW REPORT OF REVISED MANUSCRIPT

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Reviewer performed review: 2021-11-19 16:17

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Scientific quality	[<input checked="" type="radio"/>] Grade A: Excellent [<input type="radio"/>] Grade B: Very good [<input type="radio"/>] Grade C: Good [<input type="radio"/>] Grade D: Fair [<input type="radio"/>] Grade E: Do not publish
Language quality	[<input checked="" type="radio"/>] Grade A: Priority publishing [<input type="radio"/>] Grade B: Minor language polishing [<input type="radio"/>] Grade C: A great deal of language polishing [<input type="radio"/>] Grade D: Rejection
Conclusion	[<input checked="" type="radio"/>] Accept (High priority) [<input type="radio"/>] Accept (General priority) [<input type="radio"/>] Minor revision [<input type="radio"/>] Major revision [<input type="radio"/>] Rejection
Peer-reviewer	Peer-Review: [<input checked="" type="radio"/>] Anonymous [<input type="radio"/>] Onymous



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statements

Conflicts-of-Interest: [] Yes [Y] No

SPECIFIC COMMENTS TO AUTHORS

We appreciate the authors' hard work for the revision, and they replied all the comments of our team. Our team has no other comments on this manuscript.