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PEER-REVIEW REPORT

Name of journal: World Journal of Hepatology

Manuscript NO: 52988

Title: N-acetylcysteine and Glycyrrhizin combination: benefit outcome in a murine model of acetaminophen-induced liver failure.

Reviewer's code: 00573188
Position: Editorial Board
Academic degree: PhD
Professional title: Senior Scientist
Reviewer's Country/Territory: Spain
Author's Country/Territory: Belgium

Manuscript submission date: 2019-11-28
Reviewer chosen by: AI Technique
Reviewer accepted review: 2019-11-29 05:59
Reviewer performed review: 2019-12-04 20:07
Review time: 5 Days and 14 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [Y] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [ ] Grade B: Minor language polishing [Y] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[ ] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

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SPECIFIC COMMENTS TO AUTHORS

Manuscript Title: N-acetylcysteine and Glycyrrhizin combination: benefit outcome in a murine model of acetaminophen-induced liver failure. Authors: Minsart et al. This paper presents interesting results concerning the potential use of a combination of N-acetylcysteine (NAC) and glycyrrhizin (GL) to treat acute liver failure induced by acetaminophen (APAP), expanding the therapeutic window and increasing survival of the patients. However, the paper needs extensive rewriting to properly explain the election of markers for liver failure or the figures themselves. Also the English needs revision at certain points of the text. Main comments: 1- What is the reason to choose the 500 mg/kg dose? Other authors have used lower doses to obtain the same outcome. 2- Why did you choose to work only with female mice? Wouldn't be interesting to compare effects in males vs females? Some effects may depend on gender and it is interesting to explore such a point in this particular case. 3- Are you measuring ALT concentration or activity? This is a very important point that needs clarification. It would be interesting to see also AST levels of LDH levels. 4- HMGB1 is a nucleocytoplasmic enzyme that changes its subcellular localization and interaction partners in disease. In my opinion, it will be important to know whether the nucleocytoplasmic distribution of HMGB1 is changed by the drugs you use, and immunoblotting would be the best way to follow such putative changes. Also, it would be nice to see if the interaction with some of its partners is altered. 5- Is there any specific reason to used animals that are under fasting conditions? If so, please clarify. 6- Please, remember that GSH (the reduced form of glutathione) is a metabolite, not an enzyme. Thus, what you are measuring is the levels of this metabolite. In addition, knowing whether the oxidized form GSSG is increased, and hence the GSH/GSSG ratio modified, is key to understand the impact that the drugs you use may have on the recovery of the normal function of metabolic pathways that are

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affected by APAP intoxication. 7- How was the quality of the RNA analyzed? Please, explain in the Methods section. 8- I understand that the sequence of the primers used for RTqPCR might be the property of a company, but do they certify that the efficiency is 2? In my opinion, you should use more than just a reference gene in any RTqPCR studies and use calculation methods that take into account possible variations in both the gene of interest and the reference gene during the treatments (e.g. Pfaffl method). 9- Along the text there are some sections where it is not clearly stated whether the treatment is carried out together with APAP or after APAP intoxication. Please, clarify in the text (e.g. Figure 3). 10- I do not think that effects of the drugs on APAP metabolism are correctly reflected as "interference". Please, rewrite. 11- Figure 7. NAC +GL lead to an increase in glutathione levels, but do not restore control levels. 12- At some point in page 10, the expression of human CYP2E1 is mentioned, when your samples are of mice. Please, correct accordingly, since RTqPCR primers should be from the mouse sequence. 13- It would be possible to measure NAPQI metabolite in your liver samples? What about the protein-adduct content? Those parameters would be of great interest and provide additional explanations for the combined effects of the drugs. 14- I do not think that rats can be defined as a bad model for APAP-induced liver failure, since these models has been proven useful by many authors. Please, remove such as statement of the Discussion section. 15- Figure legends, should be rewritten and improved in many cases. Also, I wonder why data regarding 2h are missing in Figure 1 panels and why GSH (a metabolite not an enzyme) levels are evaluated at different times than other parameters. 16- Figures 2, 3 and 4 should clearly state that there is coadministration of the drugs. 17- Figure 3 do not shows quantification of nuclear HMGB1 labeling, neither serum values of that protein. 18- Figure 4 lacks information regarding GSH levels, serum HMGB1 and quantification of nuclear HMGB1 staining. 19- Figure 5 lacks GSH levels and nuclear HMGB1 staining, plus its quantification. 20- The legend of Figure 7 should be completely

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rewritten and the statistical analysis of panels A and B recalculated. In panel A, NAC + GL seem to induce a significant decrease in expression of CYP2E1. Panel B, shows a decrease in GSH levels by GL administration, as well by NAC+GL. Panels C and D should state clearly that they are showing effects of the drugs after APAP administration. Moreover, the presentation of all these results should be improved in the Results section and appropriately discussed under Discussion.

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PEER-REVIEW REPORT

Name of journal: World Journal of Hepatology

Manuscript NO: 52988

Title: N-acetylcysteine and Glycyrrhizin combination: benefit outcome in a murine model of acetaminophen-induced liver failure.

Reviewer's code: 02870635
Position: Peer Reviewer
Academic degree: PhD

Professional title: Professor

Reviewer's Country/Territory: China

Author's Country/Territory: Belgium

Manuscript submission date: 2019-11-28

Reviewer chosen by: AI Technique

Reviewer accepted review: 2019-12-03 00:58

Reviewer performed review: 2019-12-09 07:59

Review time: 6 Days and 7 Hours

Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [Y] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [ ] Grade B: Minor language polishing [Y] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [ ] Major revision [Y] Rejection
Re-review	[ ] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

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SPECIFIC COMMENTS TO AUTHORS

This manuscript mainly focused on the study of the protection of glycyrrhizin (GL), and the combined given of GL and NAC against acetaminophen-induced liver injury. The study was designed too simple and no new hypotheses.

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PEER-REVIEW REPORT

Name of journal: World Journal of Hepatology

Manuscript NO: 52988

Title: N-acetylcysteine and Glycyrrhizin combination: benefit outcome in a murine model of acetaminophen-induced liver failure.

Reviewer's code: 04424227
Position: Editorial Board
Academic degree: MD, PhD

Professional title: Assistant Professor

Reviewer's Country/Territory: China

Author's Country/Territory: Belgium

Manuscript submission date: 2019-11-28

Reviewer chosen by: AI Technique

Reviewer accepted review: 2019-12-04 07:58

Reviewer performed review: 2019-12-20 07:33

Review time: 15 Days and 23 Hours

Scientific quality	[ ] Grade A: Excellent [Y] Grade B: Very good [ ] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [Y] Major revision [ ] Rejection
Re-review	[ ] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

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SPECIFIC COMMENTS TO AUTHORS

The manuscript by Charlotte et al. demonstrated that N-acetylcysteine/glycyrrhizin combination significantly increased decreased the liver necrosis score and improved the survival during acetaminophen-induced liver injury in mice. Overall, the results are interesting, but there is room for improvement. 1. The unit of "12000 rpm" should be changed into " ×g". 2. "-20°" should be "-20°C". 3. Abbreviations such as GSH , etc. should be given the full name when used for the first time. 4. The English of your manuscript must be improved before resubmission. I suggest that you obtain assistance from someone whose native language is English. 5.If results are expressed as mean ± SEM, the bar in the figures shouldn't be so high. Statistical differences are not necessarily significant. Statistical expression "* vs. 0, aP<0.05; ** vs. 0, bP<0.01" are not standard. 6. The presentation and format of all figures are not standard enough. Please check and correct it thoroughly. 7. Why choose female mice? Please explain it? 8. References are insufficient, and should be updated appropriately. Hepatotoxicity, liver injury, ELISA, and RT-qPCR should be further explained. Please refere to relevant literatures: e.g. Journal of Pharmacological Sciences, 2016, 130:94-100; British Journal of Pharmacology, 2017, 174:2818–2831; J. Agric. Food Chem. 2019, 67, 2856−2864.

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PEER-REVIEW REPORT

Name of journal: World Journal of Hepatology

Manuscript NO: 52988

Title: N-acetylcysteine and Glycyrrhizin combination: benefit outcome in a murine model of acetaminophen-induced liver failure.

Reviewer's code: 00038362
Position: Peer Reviewer
Academic degree: PhD

Professional title: Associate Professor

Reviewer's Country/Territory: United States

Author's Country/Territory: Belgium

Manuscript submission date: 2019-11-28

Reviewer chosen by: AI Technique

Reviewer accepted review: 2019-12-02 14:53

Reviewer performed review: 2019-12-20 19:40

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Scientific quality	[ ] Grade A: Excellent [ ] Grade B: Very good [Y] Grade C: Good [ ] Grade D: Fair [ ] Grade E: Do not publish
Language quality	[ ] Grade A: Priority publishing [Y] Grade B: Minor language polishing [ ] Grade C: A great deal of language polishing [ ] Grade D: Rejection
Conclusion	[ ] Accept (High priority) [ ] Accept (General priority) [ ] Minor revision [ ] Major revision [Y] Rejection
Re-review	[ ] Yes [ ] No
Peer-reviewer statements	Peer-Review: [Y] Anonymous [ ] Onymous Conflicts-of-Interest: [ ] Yes [Y] No

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SPECIFIC COMMENTS TO AUTHORS

In this manuscript, the authors investigate the effectiveness of Glycyrrhizin given alone or in combination with NAC as an antidote for the treatment of acetaminophen (APAP) intoxication. Another similar study was carried-out and published using rats. The authors claimed that this work is innovative since rats are not a good animal model for APAP toxicity. This is not an accurate assertion. Rats are more resistant to APAP hepatotoxicity, but many of the mechanistic features are similar between both species of rodents. Rats respond to APAP similar to mice, but at higher doses. It could be argued that their selection of female mice for conducting the present study was equally questionable since female mice, like rats, are highly resistant to APAP hepatotoxicity. This is evidenced by their selection of a dose of 500mg/kg in PBS vehicle, which is a very high dose. In the absence of significant mechanistic insights on the mode of protection of Glycyrrhizin, this manuscript offers very little new information from what we already know from the rat study. In fact, another published study in mice provides mechanistic insights into the mode of Glycyrrhizin protection that the current manuscript lacks.