Dear Lian Sheng Ma
Editor in chief
Word Journal of Gastroenterology

We are sending to you the Point by point response to reviewers and a new version of the manuscript title: **NRF2 AND SNAIL-1 IN THE PREVENTION OF EXPERIMENTAL LIVER FIBROSIS BY CAFFEINE by Gordillo et. al.**

Please note that the new version contains all changes in bold lettering and underlying. We really appreciate the comments to the reviewers and we are certain to that they have contributed significantly to enrich our manuscript. We have modified text redaction trying to achieve a paper more accurate and comprehensible, attending all corrections by the reviewers.

Best regards
Juan Armendariz PhD
Corresponding author

Point by point response to reviewers

Revisor 1:

Minor points:

The target cell of anti-fibrotic caffeine remains to be identified. Caffein could act protective directly on hepatocytes. In this case reduced HSC activation would be an indirect effect due to reduced hepatocyte injury which triggers HSC activation. Alternatively caffeine could act anti-fibrotic by directly inhibiting HSC activation eg. by down regulation of pro-fibrotic genes specifically in HSC. However, this would not explain decreased ALT values in caffeine treated mice. Authors should discuss potential target cells on the basis of current literature.

Response

We appreciate the commentary of this reviewer and, therefore, a pertinent sentence regarding this issue was included in discussion section. (pag 15).

Heading for 3.8: correct Snai1 by Snail-1.

Response

This has been done. We have gone through the entire document and corrected all the queries. Also, we took care of grammar errors.

The English needs some minor improvement

Response

We have carefully revised the manuscript and substantially improved the document´s writing.

Revisor 2

Major points:

The authors mentioned in the discussion that our results suggest that CFA displays beneficial effects and could prevent HSC activation and perpetuation. It is unclear HSC are affected by caffeine directly or indirectly (secondary)?

Response

We have included in discussion section a paragraph where we hypothesized the mechanisms by which caffeine could prevent hepatocyte dead and HSC activation (pag 15).

To explain NRF2 and Snail-1 in the prevention of liver cirrhosis the authors should confirm which types of cells are expressing NRF2 and Snail1.

We have included in discussion section several references regarding that Snail-1 is also expressed in hepatocytes and colangiocytes and NRF2 in kupffer cells and hepatocytes. However, we are aware of the need to perform experiments like double labeling with two different markers to determine the cells expressing these transcription factors. On the other hand, we strongly feel we have complied in good faith with most of this reviewer´s queries.

Minor points:

In table 1, authors only checked the body weight. I think authors should check liver weight/body weight as most of the manuscript has done.

Response

Although it is important to correlate the body weight vs. liver weight, the body weight *per se* indicate the general health status of a given organism. The significant loss of weight observed in the groups with liver damage without CFA indicate a worsening in the overall health of these animals as compared with CFA-fed animals.

In table 2, authors only checked the serum levels of AST and ALT. Authors should check other markers such as ALP bilirrubin etc. }

Response

Although other molecules such as ALP and bilirrubin are increased in the presence of liver damage, AST and ALT are the most representative markers of hepatocyte death. Also, they stand for *bona fide* indicators to follow the course of liver disease.

Revisor 3:

Major concerns:

Was the objective of this study to investigate caffeine effects on liver fibrosis or cirrhosis? These two stages of liver disease have distinct pathology and should have been carefully defined and specifically characterized. Based on the manuscript, it seems TAA and BDL inducing fibrosis rather than cirrhosis in the present study.

Response

We completely agreed with this reviewer´s comment, and we apologize for the confusion this may have caused. Now, we specified that these two

experimental models did induce (at the time of our studies) liver fibrosis. Thus, this term has been clearly spelled out throughout all the manuscript.

The authors suggest that caffeine upregulates NRF2 and downregulates Snail1 as potential mechanisms to prevent HSC activation and the consequent fibrogenesis. However, there is no evidence that the altered NRF2 and Snail 1 protein levels are HSC specific.

Response

We appreciate this reviewer´s comments. The discussion on this issue has been extended. Indeed, we are not disclosing that Snail-1 and NRF2 are specific transcriptional factors of HSC. There is a great deal of evidence, along with our own data showing Snail-1 as a key player in the regulation of the liver fibrosis, consequently involved in HSC activation. To this end, we have included in discussion section several references regarding that Snail-1 is also expressed in hepatocytes and colangiocytes. In this paper we are reporting that Snail-1 expression drop correlates with HSC activation decrease and with less fibrosis. However, we are aware of the need to perform experiments like double labeling with two different markers to determine the cells expressing these transcription factors. On the other hand, we strongly feel we have complied in good faith with most of this reviewer´s queries.

Furthermore the SOD and CAT activities correlate well with their mRNA levels only in the BDL model, suggesting the protective effect of caffeine, at least in the TAA model could be independent of NRF2 pathway. This should be further studied and discussed.

Response

It has been discussed already in discussion section. CFA-treated rats showed higher levels of CAT in BDL+CFA group, that could be explained by the type of substrate which is hydrogen peroxide. SOD catalyzes O_2^- dismutation into O_2 and H_2O_2. In contrast, CAT catalyzes decomposition of H_2O_2 into O_2 and H_2O. Considering this, we assume that CAT was much higher in BDL+CFA group, due to accumulation of H_2O_2 at 4 weeks of treatment by SOD action.

Liver weight, liver to body weight ratio and the food intake should be reported in addition to the body weight.

We know that it is important to correlate the body weight vs. liver weight, however we think that the body weight *per se* indicate the general health status of a given organism. The significant loss of weight observed in the groups with liver damage without CFA indicate a worsening in the overall health of these animals as compared with CFA-fed animals.

Minor concerns:

Aim: needs to be rephrased. If you don´t know “whether” caffeine prevents cirrhosis, how do you study the mechanisms?

Response

The entire Abstract section has been revamped and grammar corrected

Please avoid redundant use of sentences. For example, in the abstract, “Caffeine increased SOD and CAT expression presenting a strong correlation between mRNA and activity” means the same as “Expression of SOD and CAT was greater in animals treated with caffeine founding a strong correlation between mRNA expression and enzyme activity.”

Response:

Thank you for the observation, we have taken into consideration your comments and we have improved the entire document. The entire Abstract section has been revamped and grammar corrected

Revisor 4

It is a well written and conducted study. There are some typographical errors.

Response:

Thank you for your comments, we have carefully revised the manuscript and substantially improved the document´s writing.