Author’s response to reviews

Title: Protective and Therapeutic Effects of Nanoliposomal Quercetin on Acute Liver Injury in Rats

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Author’s response to reviews:

Dear Editor,

Thank you for arranging a timely review for our manuscript entitled “Protective and Therapeutic Effects of Nanoliposomal Quercetin on Acute Liver Injury in Rats”. We would like to express our sincere appreciation for the reviewers’ careful reading and helpful comments. Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully, responded to these suggestions point-by-point, and have made correction in the manuscript accordingly which we hope meet with approval. The responds to the reviewer’s comments are described below.

Konstantinos DiMas (Reviewer 1): In this manuscript authors study the effect liposomal quercetin on liver injury of rats. Authors show some data supporting that the liposomal quercetin may show a very slight improved effect against liver injury as compared to free quercetin but the manuscripts suffers from major flaws.

-Authors did not use the appropriate statistics for the analysis of the results. ANOVA with appropriate post hoc analysis and not ttest should have been used.
Response: Many thanks for the reviewer’s suggestion. Your comments are very important for our manuscript. Indeed, for the statistical analysis of the results we used one-way ANOVA and Holm-Sidak’s multiple comparison tests for post-hoc analysis. (Statistical analysis section, line 17-19, page 7)

-A control is missing: authors should have included liposomes among the treated groups of rats.

Response: Thank you very much for your suggestion. In our project, the main composition and formula were optimized by orthogonal design. The optimal formula was used in this study. We prepared nanoliposomes with different concentration of quercetin and tested the characterization in vivo experiments. Given nanoliposomes, pure quercetin and nanoliposomal quercetin respectively, rats were detected the time and organ distribution of quercetin in vivo to testify its targeting index. In hepatic-injured models, rats were randomly divided into normal control, nanoliposomes, quercetin, or nanoliposomal quercetin group, with different doses of quercetin. In this manuscript, with the dose of 50mg/kg body weight according to quercetin, nano-modified quercetin was more effective than pure quercetin in the treatment of acute liver injury in rats. That’s the main data. (Methods section, line 4-7, page 6)

-In materials and methods: Is it a common approach the one they followed to induce liver injury? A reference should have been include herein. They also don't mention the sex of the rats.

Response: The liver injury model (mentioned in references [16],[17],[18]and[19]) in our study is simple and stable. We use carbon tetrachloride and other composite factors such as a high-fat, high-cholesterol, and low-protein diet as well as alcohol drinking, which can induce liver injury in animals with pathological results very similar to human chronic liver diseases. (Discussion section, line 1-6, page 11)

All the animals we used are healthy Sprague-Dawley (SD) rats with half male and half female. (Methods section, line 6, page 6)

-Autors did not adequately characterized the liposomes: at least a stability study vs time and temperature should have been preformed. Authors don't mention as well the constitution of the liposomes. I don't understand why authors name them "nanoliposomes". The widely used term for these liposomes is Small Unilamellar Vesicles (SUV).

Response: The samples were stable and had no stratification after being placed at room temperature for three months, and were kept in the form of freeze-dried powder for a long time. Here nanoliposomes refers to liposomes within 200 nm. There is no actual difference between “nanoliposomes” and “Small Unilamellar Vesicles” in our study. (Methods section, line 14-22, page 5)
Guzmán Alvarez (Reviewer 2): Preparation and determination of nanoliposomal quercetin; Please describe with details this section, there are no details of this preparation and characterization because it is the key of this work, Also include the detail of the quantification of quercetin in the nanoliposomes, because that is determinant to see the doses in the in vivo experiment. Please include the chromatograms in the supporting information (that also is empty, there are not extra information in these documents).

Response: We are very grateful for your suggestion, which will make this article more perfect. We have included more references in the manuscript.

The methods of preparation and determination of nanoliposomal quercetin in our group have been used for several years. The main composition and formula were optimized by orthogonal design. Briefly, liposomes, containing with quercetin were prepared from phosphatidylcholine, phosphatidylserine and cholesterol (molar ratio: 5:1:1) by a modification of the thin film evaporation method. The ethanol in mixed solution was removed under reduced pressure at 37°C by rotary evaporation to form a thin solid film. The lipid film was then hydrated with 5% glucose solution at 37°C by rotation to form a light yellow suspension. With sonication and high-pressure homogenization, the suspension was then passed through a filter membrane (0.2 μm) to remove the nonincorporated drug, and yielded nanoliposomal quercetin with high encapsulation efficiency (91.18±0.78%). The nanoparticles were stable and had no stratification after being placed at room temperature for three months, and were kept in the form of freeze-dried powder for a long time. Before experimental use, the prepared powder of nanoliposomal quercetin was well resuspended in 0.9% normal saline using ultrasound treatment to obtain a suspension of nanoliposomal quercetin with required concentration. The listed dose of liposomal quercetin is based on the content of quercetin. (Methods section,line 12-20,page 5)

Treatments in hepatic-injured rat models

In line 48 page 6, the author describes the dosage, should be reported as an mg/kg body weight, and also more clear if you use mmol/kg body weight.

The dose using pure quercetin was 50mg/kg body weight, (that is correct?). In which volume?, and also how the authors make that with the nanoliposomal (please explain that), also correlate this with the nanoparticle charge of 5mg/ml.

Response: The dose using pure quercetin was 50mg/kg body weight in 0.2 mL, the same as about 0.2mmol/kg body weight. The freeze-dried powder of nanoliposomal quercetin was resuspended in 0.9% normal saline with quercetin 5mg/0.2 mL to reach therapeutic efficacy. (Methods section,line 22,page 6)

About ethics committee, we encourage to introduce in the text the numbers or codes for the approved protocols.
Response: About ethics committee, the study protocol was approved by the Ethics Committee of Xiangya Hospital of Central South University (reference: 201503239). (Methods section,line 8-10,page 6)

In line 31-34 pag 8, the authors said that the Quercetin, present in fruits and vegetables, is one of the most common flavonoidal compounds, with non-toxic, non-lethal, non-carcinogenic, non-teratogenic and non-mutation effects in humans. Should be said, low toxic effect, because it depends on the doses, there are not non toxic compounds, and toxicity includes lethality, carcinogenicity, teratogenicity mutagenicity. Those are the definitions of these parameters in toxicology, then this is a toxicology journal, then those terms need to by according to this.

Response: Thanks for your pertinent suggestion. Quercetin is one of the most common flavonoidal compounds, with no toxic effect at an oral dose up to 2000 mg/kg body weight[25-26]. Actually, we tested an oral dose of 10g/kg body weight of quercetin in the acute and subacute toxicity test. The rats in each group were generally in good condition. There were no significant differences of body weight and food intake between the male and female rats when compared with the control group. And No statistically significant differences were found in blood routine, liver function, renal function and histopathological examination. Quercetin may be considered as relatively safe and non-toxic. (Discussion section,line 20-21,page 11)


In Table 1 please fix the line of the Nanoliposomal quercetin Treatment

Response: We fixed the line of the Nanoliposomal quercetin Treatment in Table 1. (Results section,line 2-3,page 18)

It will be appreciated if the author includes in the discusión something about the matrix in the natural origins of quercetin, and if that matrix could help the hepatic absorption, also the effect of pure quercetin was very good indid. Please incorporate some references about those issues.

Response: Your suggestion is very important and interesting. This is really a good question. Unfortunately, we didn’t study anything about the matrix in the natural origins of quercetin. We also wander if the matrix could help the hepatic absorption. The further study will help us to know. (Discussion section,line 18-21,page 13)
We tried our best to improve the manuscript and made some changes in the manuscript. We appreciate for Editors/Reviewer’s warm work earnestly, and hope that the correction will meet with approval.

Thank you for your consideration of our manuscript.

Yours sincerely,

Mingmei Liao