Author’s response to reviews

Title: Anti-inflammatory and nephroprotective activity of Juglans mollis against renal ischemia–reperfusion damage in a Wistar rat model

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Responses to reviewers

Yifei Zhong (Reviewer 1)

In this study, the authors examined the renal protective role of Juglans mollis (JM) in ischemic reperfusion rats (I/R). The authors found that JM attenuated I/R induced renal injury and oxidative and stress markers in the rats. The findings are new for JM and might be helpful as a preventive measure in patients who are susceptible for AKI. However, there are several concerns as below:

1) Since JM was given prior to injury, this study only tested the preventive effects but it would be interesting to determine whether JM still has effects after injury occurs.
Our research group seeks strategies to prevent or improve outcomes after ischemia–reperfusion (I/R) injury, which often occurs in organs involved in the transplantation process. Such information will be helpful in limiting the damage induced by I/R injury during the organ procurement process. This is why our strategy focuses on identifying chemical compounds or plant extracts that can diminish the damage to tissue caused by oxidative stress and inflammatory processes in the pretransplant phase, with the aim of improving the viability of the graft (Casillas-Ramírez A, et al., 2017; Cura-Esquivel I, et al., 2018; Torres-González L, et al., 2018). Therefore, the research study presented here did not examine the effects of the extract after injury.


2) I am not clear whether JM was given continuously after injury. This needs to be clarified.

We have clarified this in the experimental design section of the Methods section (Page 5, paragraph 6, line 111-112).

3) I don't understand why MDA increased by JM without I/R injury.

It has been reported that various compounds such as anthocyanins from various plants interfere with measurements at 532–540 nm, which can lead to the overestimation of MDA levels. This may explain the increase in the concentration of this mediator of oxidative stress in the JM rats. Other species of this genus such as Juglans regia, have been reported to contain these types of compounds. We have added this point to the Discussion section. (Page 12, paragraph 1, line 265-269)
4) The resolution of kidney histologic pictures is poor. The pictures of better quality need to be shown.

We now include histological images of kidneys with better resolution.

5) It would be nice to show some kidney staining results on the oxidative and inflammatory markers.

To compare the histological changes between study groups, we performed a quantitative histological evaluation of variables such as tubular necrosis, proteinaceous casts, exfoliated cells in the lumen, Bowman's space enlargement, lymphocytes in peritubular capillaries, medullary congestion, and intracellular vacuolization according to the methods of Kobuchi et al., Moosavi et al., and Mohamadi et al., with slight modifications. We found significant differences between the I/R and JM+I/R groups for histological parameters indicating I/R injury, such as tubular necrosis, medullary congestion, and proteinaceous casts.

We have clarified this in the experimental design section of the Methods section. (Page 8, paragraph 1, line 167-184), Results (Page 10, paragraph 4, line 238-241) and Discussion (Page 14, paragraph 1, line 308-311).


Houshang Najafi (Reviewer 2)

In this study, the authors investigated the protective effect of Juglans mollis extract against IR induced ARF. Administration of Juglans mollis extract improved the function of Kidney, decreased the levels of proinflammatory factors, and resulted in reduced oxidative stress and tissue injuries. Overall, data well support their hypothesis but manuscript is not well written. Nevertheless, following comments need addressed before publication.
1. Phytochemical analysis is necessary for Juglans mollis extract.

A description of the phytochemical analysis is included in the Methods (Page 4, paragraph 3, line 84-89), Results (Page 9, paragraph 2, line 194-196, Table 1) and Discussion (Page 12, paragraph 2, line 270-274) sections.

2. The manuscript is poorly written and contains too many grammatical errors and typos. The English definitely needs some work.

The revised manuscript has been edited by a professional editing company, OnLine English. https://www.oleng.com.au/

3. In materials and methods, it should be noted that unilateral or bilateral ischemia has been used?

Bilateral ischemia was performed. This point is now clarified in the Methods section. (Page 5, paragraph 5, line 110)

4. In figures 2-C, TNF incorrectly typed.

TNF is now presented correctly in Figure 2-C.

5. For statistical analysis: 1. What kind of analysis of variance has been used? 2. data on histopathologic damages are nonparametric and they can not be analyzed by parametric test.

1. The data were analyzed using one-way analysis of variance with the Tukey post hoc test for parametric data or the Kruskal–Wallis test with a Dunn post hoc test for nonparametric data. Prism software (version 6.0; GraphPad, San Diego, CA, USA) was used for the analyses. Differences between means were considered significant at p < 0.05. This information has been added to the Statistical analysis section at the end of the Methods section. (Page 9, paragraph 1, line 187-189).

2. To compare the histological changes between study groups, we performed a quantitative histological evaluation of variables such as tubular necrosis, proteinaceous casts, exfoliated cells in the lumen, Bowman's space enlargement, lymphocytes in peritubular capillaries, medullary congestion, and intracellular vacuolization according to the methods of Kobuchi et al., Moosavi et al., and Mohamadi et al., with slight modifications. (Page 8, paragraph 2, line 167-184) Data were analyzed using the Kruskal–Wallis test with a Dunn post hoc test for nonparametric data.
Prism software (version 6.0; GraphPad, San Diego, CA, USA) was used for the analyses. Differences between means were considered significant at p < 0.05. This information was added to the Statistical analysis section at the end of the Methods section. (Page 9, paragraph 1, line 187-189).

6. In materials and methods, what are the humane endpoints of the study?

This is a representative model of acute kidney damage, as shown by changes in oxidative stress markers and inflammatory processes that typically occur in patients during the pretransplantation phase (Zapata-Chavira, et al. 2017). We have added this information to the Methods section. (Page 6, paragraph 1, line 125-127)


7. In all figures, it is better to use two symbols. a. Comparison with the sham group. b. Comparison with IR group.

Two symbols are now used in all figures.

8. The discussion is unreliable and requires general rewrite.

The entire discussion has been rewritten.