Peer Review File

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Reviewer A

(1) Please provide a detailed description of the experimental methodology, including the number of animals used in each group.
Reply: We added detailed description (Page 3, line 85)
Changes in the text: line 85.

(2) For how long were the animals in different groups monitored? Please specify the IR technique performed on the animals.
Reply: We added some description (Page 4, line 89, line 90 and Fig 1;)
Changes in the text: line 89, line 90 and Fig 1.

(3) How does Astragaloside IV act as a renoprotective agent? Is it through antioxidant or anti-inflammatory effects? This is not thoroughly discussed in the manuscript.
Reply: We have modified our text as advised (Page 12, line 332-336)
Changes in the text: line 332-336.

(4) Reference 7 was not cited in the text.
Reply: We have revised the references.

(5) Reference 36 is mentioned in the text after reference 41.
Reply: We have revised the references.

Reviewer B

I've carefully read the paper proposed by Yamping Ding and collaborators about the protective effect of Astragaloside IV against irradiation-induced renal damages. Although the general presentation seems interesting, numerous technical interpretation errors, inadequate presentation of results and the absence of a solid context, limit deeply the interest and the scientific soundness of this work.
Major points required modifications:
- Bibliography is inadequate and did not support the question of the authors. For example, reference 1 and 3, and numerous others, did not match the text proposed. Never references (in this introduction) supported the increased impact of irradiation as well as the impact of irradiation on renal failure argued by the authors. The authors must modify their introduction and correctly used reference, with preference for original articles. Moreover, when only one article, or a little number, shown something this cannot be displayed as a dogma.

Reply: We have checked and updated the references item by item according to your advice.

- Protein analysis was not sufficiently well-conducted.
1/Authors analysed a lot of clived or modified proteins, but inadequately used term (for example cleaved caspase 1 for the use of an antibody detecting total caspase 1), inadequately displayed results (for example caspase 1 results must include blot markers to appreciate the size, whole blots to appreciate the clivage…). This is true for all proteins analyzed including caspase and interleukin.
Reply: The caspase-1 antibodies used in our article can display both total caspase-1 and cleavage caspase-1, and we only incubated the band with 20 kDa. Similarly, only cleaved bands of caspase3,9 were incubated. We will pay attention to retain the blot markers and the whole blots blot markers in the future. Thank you very much for your valuable advice. We will improve it in our future work.
2/ GAPDH or another loading control should be displayed for all blots. It is clear that the blots displayed in the same panel come from different membranes, so the loading control must be shown for each of them.
Reply: We have added loading control for each band from different membranes. (Figure 2,4,5,6)
Changes in the text: Figure 2,4,5,6.

3/ For cytochrome c analysis by western blots, authors must display GAPDH for mito samples and COX-IV for cyto samples to demonstrate the purity of their fractions. There is no sense to display this kind of experiment without these controls.
Reply: We have displayed GAPDH and COX-IV for mito and cyto samples. Thank you for your professional advice. (Figure 1)
Changes in the text: Figure 1.

- Histology is not informative without indication of the region of the kidney used or analysed. In addition, it will be better to have different panels presenting analyses of the various regions of the kidney to provide solid support for the conclusion, with clear identification of proximal, distal, and collecting tubules as well as cortical and medullary zones. However, all results must be analysed and comment taking account these several parts of the kidney.
Reply: We have modified our text and figure as advised (Page 8, line 233-239; Fig 7B;)
Changes in the text: line 233-239; Fig 7B;

- Ros analyses are inadequate without counterstaining to identify the region analysed. Minor points: Why the authors used DCFDA-diluent for DHE staining?
Reply: We have added the sampling region in the text (Page 5, line 129). In addition, dihydroethidium was used instead of DCFDA. We have modified it, sorry for the writing error. Thank you for your careful review (Page 5, line 132).
Changes in the text: line 129; line 132.

- In the same way, TEM analyses have no sense without indication of the regions and kind of tubule.

Reply: In the study, renal cortex were harvested, and the sections were first evaluated under an TEM at low magnification to identify representative proximal tubules and then analyzed at high magnification. The detailed descriptions have been added in the text (Page 5, line 117-120). Thank you for your professional advice.

Changes in the text: line 117-120.

- Finally, 3 samples are not sufficient to clearly assess the effect of any compounds in any situations, this sounds scientifically objectionable. In this context, I'm really surprised and puzzled by the very low level of heterogeneity in the results shown for several biochemical parameters.

Reply: In the study, 10 animals' blood were taken from each group, and then the blood of 3 animals were mixed as one sample and sent to the technology company for biochemical indicators testing.