Peer Review File

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Reviewer A

1) First, the title needs to indicate the research methodology. The authors need to tone down the current title since it is not consistent with the conclusion and seems to be overstated.

Reply: Thank you for your review comments. The title of the article is really exaggerated.
Changes in the text: The new title is "Porous Se@SiO2 nanocomposites play potential inhibition role of hyperoxaluria associated kidney stone by exerting antioxidant effects".

2) Second, the abstract needs some revisions. The background did not indicate the potential clinical significance of this research focus and its corresponding knowledge gap. The methods need to describe the number of animals, the grouping method, and the intervention in the control group. The results need to specify data on cytotoxicity outcomes and the Se concentration levels. The conclusion needs comments for the clinical implications of the findings.

Reply: Thank you for your review comments. We have made revision according to your review comments in abstract part.
Changes in the text:
Background: Nephrolithiasis seriously affect people's health with increasing prevalence and high recurrence rate. However, there is still a lack of effective interventions for the clinical prevention of kidney stones. Hyperoxaluria-induced renal tubular epithelial cell (TEC) injury is a known key factor in kidney stone formation. Thus, developing new drugs to inhibit the hyperoxaluria-induced TEC injury may be the best way.(Line 47-51)

Methods: We synthesized the Se@SiO2 nanocomposites as described previously (PMID: 29026307). The size and morphology of the Se@SiO2 nanocomposites were captured by transmission electron microscopy. Cell viability was measured by a Cell Counting Kit-8 assays. The mice were randomly divided into the following 4 groups: (I) the control group (n=6); (II) the Se@SiO2 group (n=6); (III) the glyoxylic acid monohydrate (GAM) group; and (IV) the GAM + Se@SiO2 group (n=6). The concentration of Se in the mice was measured by inductively coupled plasma atomic emission spectroscopy.

Result: The CCK-8 assays showed that Se@SiO2 nanocomposites had almost no obvious cytotoxicity on the Transformed C3H Mouse Kidney-1 cell (TCMK-1). The mice kidney Se concentration levels in the Se@SiO2 groups (Se@SiO2 6.905±0.074 mg/kg; GAM+Se@SiO2 7.673±2.85 mg/kg) (n=6)were significantly higher than those in the control group (Control 0.727±0.072 mg/kg; GAM 0.747±0.074 mg/kg) (n=6). The

Se@SiO2 nanocomposites reduced kidney injury, calcium oxalate crystal deposition, and the osteoblastic-associated proteins in the hyperoxaluria mice models.

Conclusions: Se@SiO2 nanocomposites appear to protect renal TECs from hyperoxaluria by reducing reactive oxygen species production, suggesting the potential role to prevent kidney stone formation and recurrence.

3) Third, the introduction of the main text needs to explain more the study hypothesis and why the current animal study could answer the research question. Possible clinical implications need to be discussed.

Reply: Thank you for your review comments. We have made revision according to your review comments in introduction part.

Changes in the text: We have added the following paragraph in introduction part in line 112-116.

In this study, we investigated whether Se@SiO2 nanocomposites ameliorated hyperoxaluria-induced kidney stone formation in mice models. The potential mechanisms underlying the protective effect of Se@SiO2 nanocomposites on hyperoxaluria-inducedinduced renal TEC injury were also explored. The results of this study may provide a new direction for the treatment of kidney stones.

4) Fourth, in the methodology of the main text, please describe the research design of this study and ensure P<0.05 is two-sided in statistics. Please specify the statistical test for multiple comparisons after the one-way ANOVA. The authors need to specify the variables used in the paired t test.

Reply: Thank you for your review comments. We have made revision according to your review comments in statistical analysis part.

Changes in the text: The data analysis was performed using GraphPad Prism 7 (GraphPad Software Inc., San Diego, CA, USA). The data are expressed as the mean ± standard deviation (n=6). The unpaired Student's t-test and a one-way analysis of variance were used for the comparisons. P values <0.05 were considered significant.

5) Finally, please consider to cite some related papers: 1. Chen X, Li S, Shi C, Zhang W, Liu Z, Jiang J, Zhang Y, Chen Z, Zheng B, Zhu H. Risk factors and predictors of urogenous sepsis after percutaneous nephrolithotomy for idiopathic calcium oxalate nephrolithiasis. Transl Androl Urol 2023;12(6):1002-1015. doi: 10.21037/tau-23-219. 2. Zou B, Zhou Y, He Z, Zhou X, Dong S, Zheng X, Xu R, Duan X, Zeng G. A critical appraisal of urolithiasis clinical practice guidelines using the AGREE II instrument. Transl Androl Urol 2023;12(6):977-988. doi: 10.21037/tau-22-846. 3. Zhao Y, Fan Y, Wang M, Yu C, Zhou M, Jiang D, Du D, Chen S, Tu X. Kidney stone disease and cardiovascular events: a study on

bidirectional causality based on mendelian randomization. Transl Androl Urol 2021;10(12):4344-4352. doi: 10.21037/tau-21-899.

Reply: These articles are excellent references for our study. Thank you very much.

Changes in the text: These articles have been cited in our paper at (5), (8) and (23).

Reviewer B

The paper titled "Porous Se@SiO2 nanocomposites ameliorate hyperoxaluria-induced kidney stone formation by exerting antioxidant effects" is interesting. Se@SiO2 nanocomposites appear to protect renal TECs from hyperoxaluria by reducing reactive oxygen species production. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) What are the biggest advantages and disadvantages of nanocomposites in this study? What is the most difficult technical challenge to overcome? Suggest adding relevant comparative analysis.

Reply: Thank you for your review comments. There is no innovation in our nanomaterials, just a repositioning application of previous materials.

2) In the introduction of the manuscript, it is necessary to clearly indicate the knowledge gaps and limitations of prior study and the clinical significance of this study.

Reply: Thank you for your review comments. We have indicated the knowledge gaps and limitations of prior study and the clinical significance of this study.

Changes in the text: the last paragraph introduction part: In this study, we investigated whether Se@SiO2 nanocomposites ameliorated hyperoxaluria-induced kidney stone formation in mice models. The potential mechanisms underlying the protective effect of Se@SiO2 nanocomposites on hyperoxaluria-inducedinduced renal TEC injury were also explored. The results of this study may provide a new direction for the treatment of kidney stones. We present this article in accordance with the ARRIVE reporting checklist.

3) The ROS and TUNEL detection results in Figure 4 are unclear. Suggest replacing the figures.

Reply: Thank you for your review comments. The ROS and TUNEL detection results in Figure 4 in our computer looks very clear. Maybe the problem of application program.

4) The description of some methods in this study is too simplistic, please describe in detail.

Reply: Thank you for your review comments. We have revised the methods section. Such as in Animal experimental design part.

5) There are still some weak points in this paper. It is suggested that the author increase the research of signaling pathway. This is more conducive to support the conclusions of this study.
Reply: Thank you for your review comments. In this paper, we are only making a preliminary application exploration, and we will further study the corresponding signaling pathways in the follow-up study.

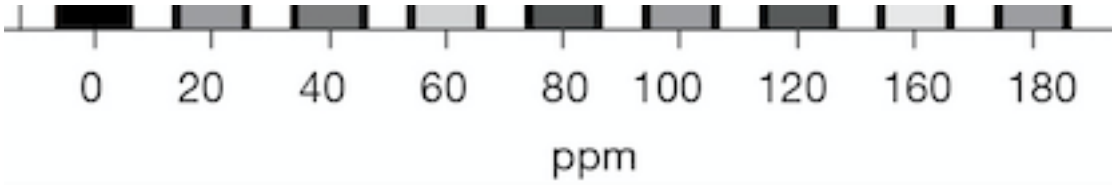
6) The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Harmine loaded Au@MSNs@PEG@Asp6 nano-composites for treatment of spinal metastasis from lung adenocarcinoma by targeting ANXA9 in vivo experiment, Transl Lung Cancer Res, PMID: 37323183". It is recommended to quote this article.
Reply: Thank you for your review comments. We have add these paper as reference.

7) What is the potential clinical importance of nanocomposites in alleviating the side effects of chemotherapy? It is recommended to add relevant content.
Reply: Thank you for your review comments. But our research did not involve chemotherapy.

Reviewer C

1. Figure 1

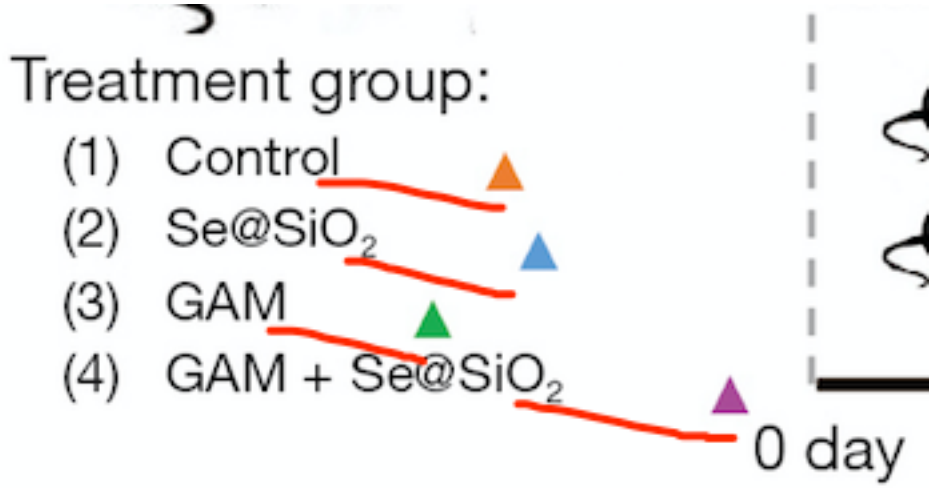
Please confirm if 'ppm' is an abbreviation. If so, please indicate its full term in the figure 1 legend, and revise it to 'PPM'.



PPM means parts per million. We have added the full term in the legend. But, it's customary to use lowercase.

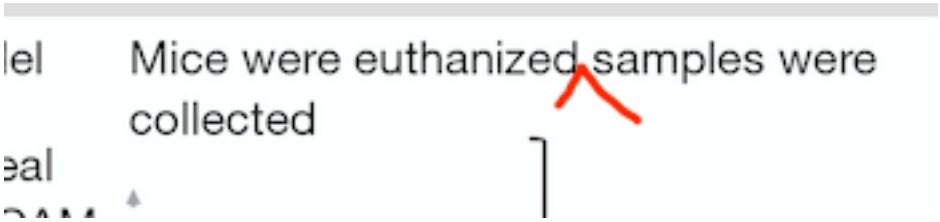
2. Figure 2

a. These four groups and colorful triangles should be in parallel. Please revise.



Sorry about this. We have rearranged these.

b. Please add a word 'and' here.

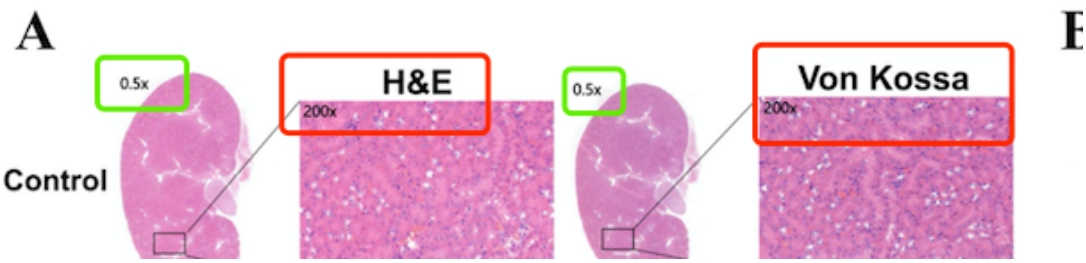


We have added "and" here.

3. Figure 3

Please confirm if the legend is correct.

Figure 3 Porous Se@SiO₂ nanocomposites inhibit hyperoxaluria-induced CaOx crystal deposition and function impairment on renal. (A) H&E staining (left, magnification ×0.5) and Von Kossa staining (right, magnification ×200) of the mice



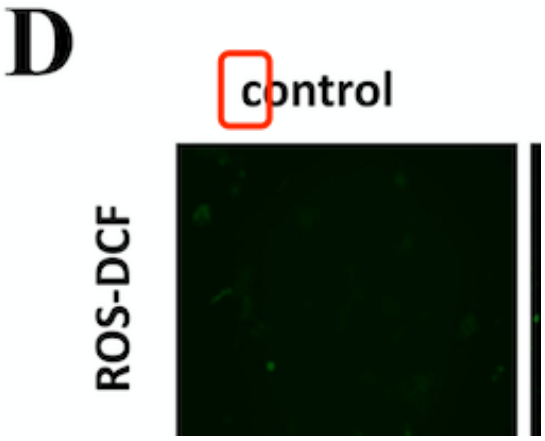
So sorry about it. We have revised the legend here, and confirm the new legend is correct.

Figure 3 Porous Se@SiO₂ nanocomposites inhibit hyperoxaluria-induced CaOx crystal deposition and function impairment on renal. (A) H&E staining (left,

magnification×0.5 for full view, magnification×200 for local zoom) and Von Kossa staining (right, magnification×0.5 for full view, magnification×200 for local zoom) of the mice kidney tissues.

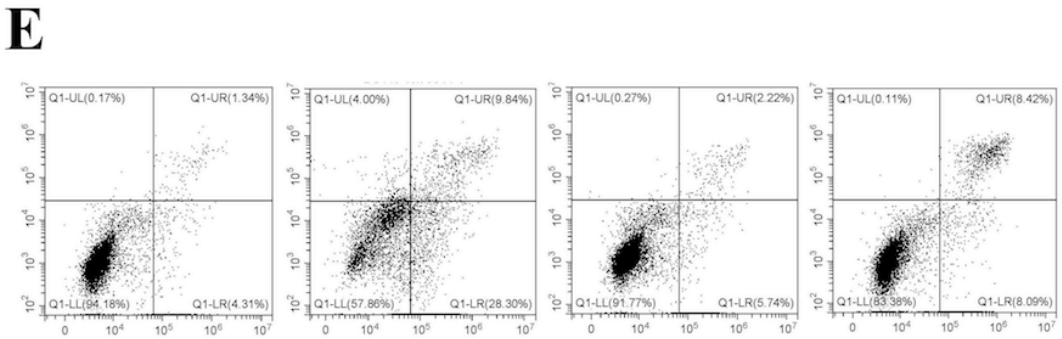
4. Figure 4

a. The first letter ‘c’ should be capitalized.

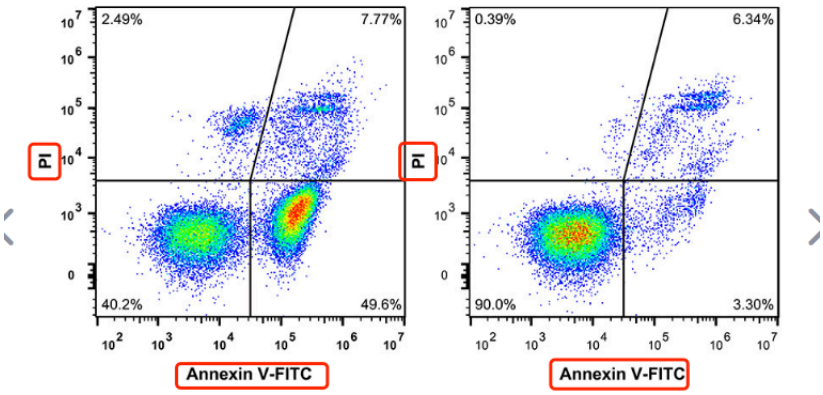


Sorry about this. We have revised it.

b. Please add the caption for the Y-axis and X-axis.



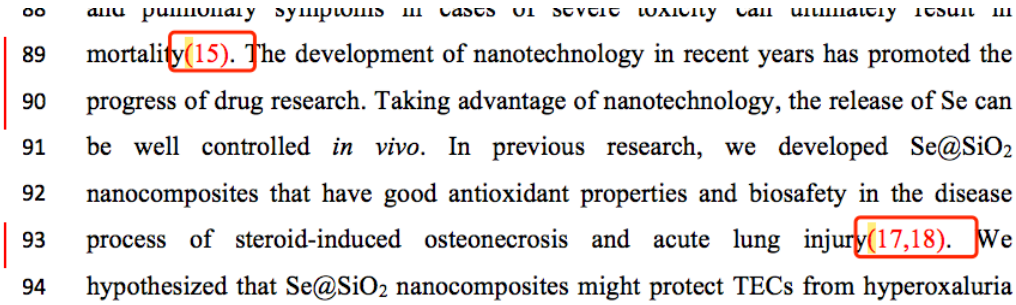
Here is an example:



Sorry about this. We have revised it.

5. Reference/citation

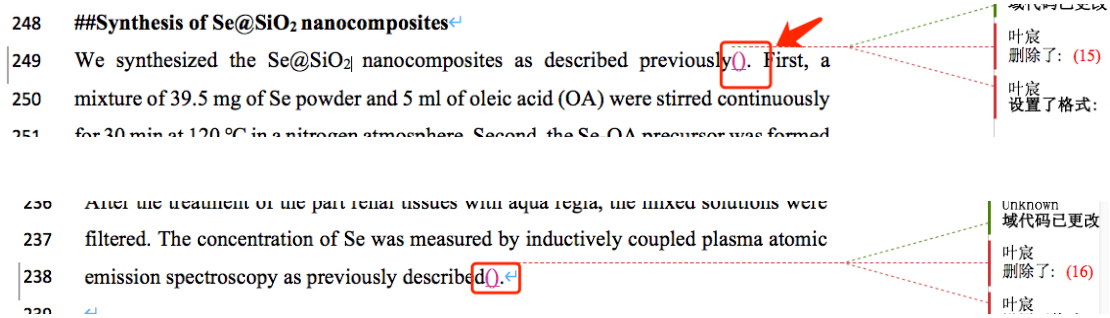
a. The citation of reference 16 is missing in the main text. It should be cited consecutively between references 15 and 17



We have refilled the missing reference in the main text.

b. Please check if citations are missing here.

Note: References should be cited consecutively and consistently according to the order in which they first appear in the text.



We have added these references in manuscript.

c. Please check if references 18 and 19 are same study. If so, please delete one of them and update the citations in the paper.

18. Zhu Y, Deng G, Ji A, et al. Porous Se@SiO2 nanospheres treated paraquat-induced acute lung injury by resisting oxidative stress. Int J Nanomedicine. 2017. 12: 7143-7152.

19. Zhu Y, Deng G, Ji A, et al. Porous Se@SiO(2) nanospheres treated paraquat-induced acute lung injury by resisting oxidative stress. Int J Nanomedicine 2017;12:7143-52.

So sorry about it. We have deleted the old references 19.

c. The authors mentioned "studies...", while only one reference was cited. Change "Studies" to "A study" or add more citations. Please revise. Please number references consecutively in the order in which they are first mentioned in the text.

Previous studies have shown that high oxalate and CaOx crystals induced the TECs to generates excess ROS, which severely damages cell structure and function through oxidation reactions with lipids, carbohydrates, proteins, and nucleic acids(28).

Sorry about this. We have changed "studies" to "a study review", because the reference

is a review.

A study review have introduced that high oxalate and CaOx crystals induced the TECs to generates excess ROS, which severely damages cell structure and function through oxidation reactions with lipids, carbohydrates, proteins, and nucleic acids(27).