Peer Review File

Protective effect of Danhong injection on retina of diabetic rats

Reviewer 1

1- The authors have spent some effort communicate their findings, although the English language is deficient and often mis-communicates the intention of the author. I highly recommend that the authors have the manuscript reviewed and professionally edited for linguistic and grammatical errors.

1- First of all, thank you very much for reading my paper so carefully and putting forward professional suggestions, which can improve the article to a higher level and benefit me a lot. I have carefully revised the article according to your opinion. I am a clinical doctor, and I really hope that I can write my findings on scientific research and communicate with more people. However, my English is not very good, and I am making great efforts to study and improve myself through continuous writing. This is my first English paper, thank you for reviewing and proposing for me, I got a lot out of it.

2- The project needs to have approval from an Institutional Animal Care and Use Committee (IACUC). This governs how ethical it is to involve the number of animals used and whether the experimental design is warranted etc. I see no mention of that in the paper. See the APA guidelines for ethical conduct in the care and use of nonhuman animals in research below: https://www.apa.org/science/leadership/care/care-animal-guidelines.pdf

2- This topic comes from the science and technology fund project of Shanghai jiaotong university school of medicine. The feeding and use of experimental animals follow the requirements of experimental animal ethics and comply with the relevant ethical regulations of animal experiments of the ninth people's hospital affiliated to Shanghai jiaotong university school of medicine. (I have added notes in the article.)

3- There needs to be a fuller description of the rats involved in this research, adherent to the Guidelines for Nomeclature of Mouse and Rat Strains: http://www.informatics.jax.org/mgihome/nomen/strains.shtml

Please mention the vendor, keeping in consideration that the Streptozotocin-Induced Diabetic Nude Mouse Model shows differences between animals from different sources.

3- Rats purchased from Shanghai silaike Experimental Animal Co., Ltd. (I have added notes in the text).

Before modeling, we reviewed relevant literatures. Previous studies have proved that the diabetes model made by this type of rats is stable and reliable, and most studies on drug intervention type adopt this type of rats, which is the reason why we choose SD rats.

As for the differences between animals from different sources, we need to continue to consult the literature for further understanding.

4- The blank intervention group was administered distilled water? Why not a standard isotonic solution like physiological saline or PBS? A hypotonic solution such as dH2O causes the cells to
burst, which is painful to animals. You should follow OECD guidelines for animal studies.

5- Related to the previous point, you should mention the solvent that was used for the danhong injection. Typically the negative control ("blank intervention") is injected with the solvent of the positive intervention so that the two groups are fully comparable.

4+5 Before the experimental design, we consulted the instructions of danhong injection, and distilled water was selected as the solvent in the production of the drug. In order to reduce the error, we selected distilled water as the blank control.

6- Observation of retinal tissue morphology (H&E): Only qualitative descriptions provided; need a more quantitative measure of morphology. For example, two independent, blinded observers should be asked to assign a score from 1-5 on various aspects like nerve arrangement, edema, etc.

6- Because we used Tunel staining to tag apoptotic nerve cells, this is an experimental method that can be used for quantitative analysis. Therefore, quantitative analysis of HE staining was not performed.

7- All figures just show one example from each group, which may be cherry-picked. At least show ~3 examples from each group.

7- In this paper, we selected a variety of experimental methods to display the morphology of the retina, such as HE, TUNEL, electron microscopy, immunohistochemistry and so on. There are already 16 images, although posting more would make the results more believable and convincing. Considering the layout, page space, publishing costs, etc., our team only agreed to let me choose the most representative one for each group and method.

8- Figure image quality is very poor in figures 1, 2 and 4. Images need to be in clear focus. Get rid of the panel numbers at the bottom ("1A", "1B", ...) and place clean text at the top of each panel not including the figure number, i.e. "A", "B", .. etc. Also, please add a scale bar at the bottom of each image showing length in micrometers.

8- The pictures have been modified according to the requirements, and calipers have been added to the pictures of the electron microscope.

8- Under TUNEL staining section, the paragraph starting with "The average number of positive cells ..." is much better described using a bar graph with overlaid scatter plot. Would be good to see the individual points rather than summary descriptors.

9- Thanks for your reasonable suggestion. We have added the scatter plot.

10- Line 25, page 1. "[...] was significantly different from" -- the word "different" here is not clear. Should use a more descriptive term like increased/decreased/etc.

10- Compared with the diabetes group and the blank control group, danhong injection group
decreased.
The paper has been revised

11- Line 28, page 1: "[...] danghong intervention group was lower than in the control group" -- which control group? have two control, the normal group and the blank intervention.

11-The paper has been revised

12- Line 29, page 2: "(this procedure was carried out by two experimenters who were not in our research group [...] under light microscope)" -- This is an unnecessary digression and adds nothing to the reader. The proper way to do this is to thank the people who performed this procedure in the Acknowledgements section of this paper.
12-Relevant sections have been deleted

13- Lines 54/55, Page 2: Need a separate subtitle called "Results" to distinguish methods from results sections.
13. Added "Results" in the corresponding section

14- Line 42, page 4: "extremely urgent" is a very strong word to use in a scientific article. Replace with something like "critical".
14. Thank you very much for the excellent proposal. The corresponding part has been replace with "critical".

Reviewer: 2
Accept as it is.