Reviewer #1:

Comments to Authors: authors explored the effect of quercetin on reduction of oxidative stress-induced senescence of nucleus pulposus-derived mesenchymal stem cells and investigated the miR-34a/SIRT1 signaling pathway. Generally, it is an interesting study, however there are some comments and questions the authors should address all were detailed below: Major corrections: • Describe the component of medium used for culturing NPMSCs, authors didn't mention the proper medium. • Authors are encouraged to provide original gel of the western blots. • Introduction needs to be summarized.

Answer：Thank you for your constructive comments and suggestions. We had already described the component of medium used for culturing NPMSCs in our article, we had already summarized our instruction to a structured abstract. We will send the organized western blots to the editor's mailbox later.

Reviewer #2:

There are a couple of points that should be considered to potentially increase the structural quality and coherence of the paper. 1. Please take into account having a structured abstract, given the nature of the paper, that should contain the following key points: Objective, Methodology, Results and Conclusion, in a brief manner 2. Regarding the "Radiographic evaluation and histological analysis" section, please correct to "as previously described" at line 9. 3. Regarding the "Effects of Que on SA-β-Gal staining and the cell cycle in NPMSCs" section, line 1, please use "of cellular senescence". After analyzing the manuscript, it can be considered for publication after making these minor changes.

Answer: This is a great opinion. We thank the reviewer for raising this issue. we had already summarized our instruction to a structured abstract. We have already corrected the "Radiographic evaluation and histological analysis" section, line 9, to "as previously described". We have already corrected the "Effects of Que on SA-β-Gal staining and the cell cycle in NPMSCs" section, line 1, to "of cellular senescence".

Editor-in-Chief review

1) The title should be indicated the data set from rat modeling, not humans. The current version of the title: "Quercetin ameliorates oxidative stress-induced senescence in nucleus pulposus-derived mesenchymal stem cells via the miR-34a-5p/SIRT1 axis," is too generic to be specific.

Response: We really appreciate for this valuable recommendation. We change the title to “Quercetin ameliorates oxidative stress-induced senescence in rat nucleus pulposus-derived mesenchymal stem cells via the miR-34a-5p/SIRT1 axis”.

2) Abstract: "CONCLUSION a. In summary, the present study provides evidence that Que reduces oxidative stress-induced senescence of NPMSCs via the miR-34a/SIRT1 signaling pathway, suggesting that Que may be a potential agent for the treatment of IDD."

3) Page 18: "Hence, it remains unknown whether Que plays a role in NPMSC senescence induced by oxidative stress via other signaling pathways, suggesting that further study is needed to explore the other mechanisms underlying the protective effect of Que on NPMSCs. Moreover, future clinical studies are needed to evaluate the effect of Que on IDD progress." What are other signaling pathways?

Response: We are so grateful for your questions. Previous studies had demonstrated that Que shows anti-inflammatory and antioxidative effects through other signaling pathways, such as NRF2, MAPK, PI3K/AKT signaling pathways. This suggests that Que does not exert its anti-inflammatory role through just only one signaling pathway. Some studies have reported that Que is an effective activator of SIRT1 [1,2] and also reduces the expression of miR-34a [3]. Thus, we focused on the miR-34a/SIRT1 signaling pathway in our present study and the results also confirmed that Que can reduce oxidative stress-induced senescence of NPMSCs via the miR-34a/SIRT1 signaling pathway. However, we did not explore whether other signaling pathways are participate in. Thus, perhaps other signaling pathways were involved.

[1]Xu D, Hu M-J, Wang Y-Q, et al. Antioxidant Activities of Quercetin and Its Complexes for Medicinal Application. Molecules. 2019;24(6).

[2]Iskender H, Dokumacioglu E, Sen TM, et al. The effect of hesperidin and quercetin on oxidative stress, NF-κB and SIRT1 levels in a STZ-induced experimental diabetes model. Biomed Pharmacother. 2017;90:500-508.

[3] Kim M, Jee SC, Shin MK, et al. Quercetin and Isorhamnetin Reduce Benzo[a]pyrene-Induced Genotoxicity by Inducing RAD51 Expression through Downregulation of miR-34a. Int J Mol Sci. 2022 Oct 28;23(21):13125.

4) Figures 2, 3, 4, 5, 6, 8, 9, 10, 11: Why did the authors not include Que (Quercetin) alone, but with TBHP (Tert-butyl hydroperoxide to trigger oxidative stress in NPMSCs) alone and Que + TBHP?

Response: Thank you for your constructive comments and suggestions. Because the purpose of this study was to investigate the effect of Que on oxidative stress injury in NPMSC, oxidative stress injury was not apparent in normal NPMSC. Therefore, in order to investigate the protective effect of Que on oxidative stress injury, we first built a model of oxidative stress injury and then explored the protective effect of Que. This grouping is also the grouping method used in the vast majority of studies so far[4,5]. Of course, it would be better if there was a Que alone group, but the current grouping results can be used to achieve our study purpose.

[4]Shi P-Z, Wang J-W, Wang P-C, et al. Urolithin a alleviates oxidative stress-induced senescence in nucleus pulposus-derived mesenchymal stem cells through SIRT1/PGC-1α pathway. World Journal of Stem Cells. 2021;13(12):1928-1946.

[5] Shao Z, Wang B, Shi Y, et al. Senolytic agent Quercetin ameliorates intervertebral disc degeneration via the Nrf2/NF-κB axis. Osteoarthritis Cartilage. 2021 Mar;29(3):413-422.

5) Page 11: "In total, 15 male SD rats (2-4 mo old and weighing 200-300 g) were randomly divided into the following three groups: Control group (n = 5), IDD group (n = 5), and Que group (n = 5)." What was the justification of 5?" What was the power of confidence in statistics? A high-power value (e.g., 0.8 or higher) indicates a greater

likelihood of detecting a true effect if it exists. Researchers typically aim for high power to ensure their study has sufficient sensitivity to detect meaningful effects. Neither Jing-feng Li, Ph.D., the statistician, nor the authors stated that.

Response: Thank you for your constructive comments and suggestions. We use Resource Equation Approach proposed by Arifin [5] in 2017 to calculate the sample size of animal experiments.

N = total number of rats, k = number of groups, and n = number of rats per group.

In our study, rats were divided into 3 groups, the sample sizes per group are:

$$N(min) = 10/3 + 1 = 4.3 = \text{rounded up to 5 animals}$$

$$N(max) = 20/3 + 1 = 7.7 = \text{rounded down to 7 animals}$$

The total sample sizes are 15-21 animals

According to the 3R principle (reduction, refinement, and replacement) in animal research, meanwhile, our research budget is tight, we select the minimum animals for the animal research.

[5] Arifin WN, Zahiruddin WM. Sample Size Calculation in Animal Studies Using Resource Equation Approach. Malays J Med Sci. 2017 Oct;24(5):101-105.

6) Page 13: "Effects of Que on MMP and ROS generation NPMSCs" – What does that mean in English?

Response: Thank you for pointing this out. It may be that our expressions are not standardized enough. We changed to “Effects of Que on MMP and ROS generated in TBHP-treated NPMSCs”.

7) Page 15: "However, Que treatment alleviated the degeneration and morphological changes in the NP and AF (Figure 12F)." Beyond morphology, any functional behavior testing existed?

Response: Thank you for your constructive comments and suggestions. This is a great opinion. Our rat intervertebral disc degeneration models were created by 21 G needle to puncture the coccygeal intervertebral disc. The rat is a reptile, and its tail coccygeal intervertebral disc does not have a load-bearing function like human disc. Most of the previous studies on intervertebral disc degeneration in rats have been performed only

morphologically. Therefore, we had only run morphology. This is a shortcoming of our research. In our future studies, we will further refine the research protocol and add functional behavior testing to it as well.

8) A schematic diagram, like graphic abstract or molecular mechanisms, should be included to enhance the manuscript's clarity.

Response: We are so grateful for your questions. In our manuscript, Figure 13 is the Schematic representation of the mode of action of quercetin.