Diabetes mellitus is a metabolic disorder that threatens the health of individuals worldwide. In the manuscript "Gambogic acid affects high glucose-induced apoptosis and inflammation of retinal endothelial cells through the NOX4/NLRP3 pathway", authors investigate the effect and mechanism of gambogic acid (GA) on the apoptosis and inflammation of human retinal endothelial cells (HRECs) under high glucose conditions.

Couple questions are required to be answered before it will be accepted.

(1) What were the roles of NOX4/NLRP3 pathway in the inflammation of diabetes mellitus? Please state in the introduction.

Reply: Thank you for your comment. We have added the roles of NOX4/NLRP3 pathway in the inflammation of diabetes mellitus in the introduction.

Changes in the text: Introduction / Paragraph 1

(2) In the introduction, it was advised to add related reference (Ann Transl Med. 2022 Feb;10(3):134) about the relationship between diabetes mellitus and inflammation.

Reply: Thank you for your comment. We have added the related reference in the introduction.

Changes in the text: Introduction / Paragraph 1

(3) What is the meaning of "Gambogic acid (GA) is one of the main active components of GA" in the introduction? Please state clearly.

Reply: Thank you for your comment. Garcinic acid (GA) is one of the main active components extracted from the dried resin secreted by Garcinia tenuifolia, a plant of the family Garcinaceae. We have revised it in the introduction.

Changes in the text: Introduction / Paragraph 2

(4) Whether the gamboflavin was the same to GA? Please state clearly.

Reply: Thank you for your comment. I'm very sorry that this is our clerical error. We have revised it in the manuscript.

Changes in the text: Methods / Paragraph 2 and 4; Results / Paragraph 2; Discussion / Paragraph 2.

(5) How to determine the concentrations of GA? The 0.5, 1, 2, 5, 10, 20, 50, and 100 μM of GA was not multiple ratio.

Reply: Thank you for your comment. We set the GA concentration (0.5, 1, 2, 5, 10, 20, 50, and 100 μM) gradient to screen the appropriate GA intervention concentration. This study found that compared with the control group (0 μM) Group comparison, 20 μM The activity of cells treated with GA at M concentration decreased significantly (P<0.05). Further concentration gradient experiment screening 15 μM GA treatment of M concentration is

the optimal intervention concentration, so 15 μ M concentration of GA treated cells.

Changes in the text: None.

(6) The t-test was used to perform statistical analysis between two groups. There were over two groups in the study. How to perform statistical analysis? Please check again.

Reply: Thank you for your comment. Single factor analysis of variance was used for comparison among groups. We have added it in the Methods.

Changes in the text: Methods / Paragraph 10.

(7) The ASC was the main components of NLRP3 inflammasome. It was better to test the expression of ASC.

Reply: Thank you for your comment. We have added it.

Changes in the text: Results / Paragraph 4 and 5; Figure 4, 5A and 5C.

(8) Please perform statistical analysis about the Western blot.

Reply: Thank you for your comment. We have added it.
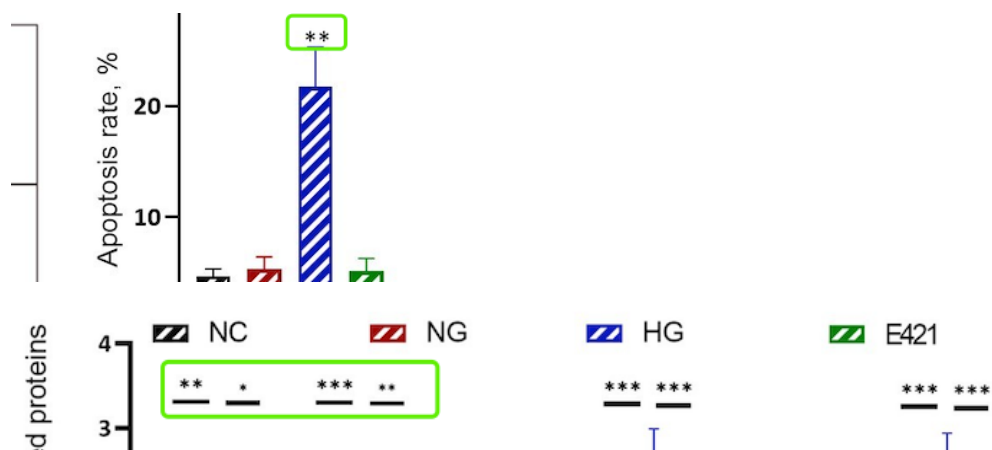
Changes in the text: Figure 1B, 3B, 4, 5A and 5C

1. Table 1:

Please indicate the full name of "IL", "TNF-α" in table footnote.

Reply: Thank you for your comment. We have added it.

2. Figure 1:

Please indicate the meaning of ** in Figure 1A and *, **, *** Figure 1B legend.

Reply: Thank you for your comment. We have added it.

3. Figure 3:

Please indicate the meaning of *, **, *** Figure 3B legend.

Reply: Thank you for your comment. We have added it.

4. Figure 4:

1) Please indicate the meaning of **, *** Figure 4 legend.

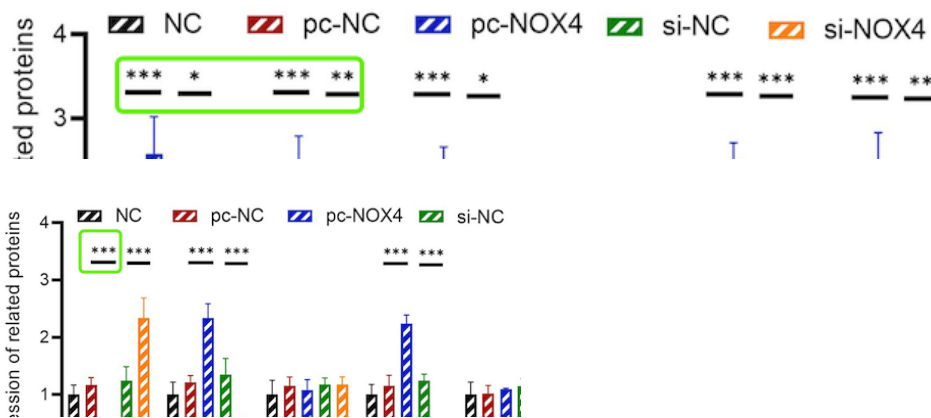Reply: Thank you for your comment. We have added it.

2) Please indicate the full name of "ASC" in the legend.

Reply: Thank you for your comment. We have added it.

5. Figure 5:

1) Please indicate the meaning of *, **, *** Figure 5A legend and *** in Figure 5B legend.

Reply: Thank you for your comment. We have added it.

2) Please indicate the full name of "ASC" in the legend.

Reply: Thank you for your comment. We have added it.