Peer Review File

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Reviewer A

Comment 1: The description of some methods in this study is too simplistic, please describe in detail.
Reply 1: Thank you so much for your valuable comments. I have incorporated them into the methods section of the article and highlighted them in green font.
Changes in the text: We have modified our text as advised (see page 8,9, line139-144, see page 9,10, line161-162)

Comment 2: What is the potential application value of puerarin in clinical practice? What is the basis for selecting the concentration of puerarin in this study? Is the dosage safe in clinical practice? Please provide literature support.
Reply 2: Thank you for your question, which is of great significance for future clinical applications. Firstly, in comparison with other flavonoids, puerarin is readily available and more affordable in our southern region of China. It has demonstrated clear therapeutic effects in various anti-inflammatory, antioxidant, and immune-related diseases. Our selection of puerarin is based on previous research screening that suggests its potential as a protective factor against environmental disturbances in children with GD (manuscript references for details: 2), thereby preventing the occurrence and progression of GD. The concentration used in this study was determined through extensive literature review and preliminary experiments (manuscript references for details: 8-9,17-19,20,22,24-27,58). Currently, our main objective is to verify whether puerarin can serve as an environmental protective factor for GD by examining its impact on GD mouse models and elucidating its underlying mechanism of action. Based on these findings, we will further explore the possibility of utilizing puerarin as a therapeutic drug for GD patients while determining the appropriate dosage.
Changes in the text: We have modified our text as advised (see page 5, line 64-66, page 15, line 266-271)

Comment 3: There are still some weak points in this paper. It is suggested that the author increase the inhibitor or agonist of signaling pathway. This is more conducive to support the conclusions of this study.
Reply 3: Thank you for your valuable advice. I also realized the same thing. Our next step plans to improve the recovery experiment by increasing the inhibitor or agonist of the signaling pathway for this study. However, it is important to note that the animal

model requires a minimum of 10 weeks for modeling, or additional exploration is needed to determine the temporal concentration gradient of puerarin in the cell model. Therefore, a duration of 3 weeks seems insufficient. If feasible, we could potentially extend the modification time appropriately in order to further enhance the relevant experiments in this regard.
Changes in the text: We have modified our text as advised (see page 18, line 323-326)

Comment 4: There are many drugs and genes that regulate the inflammation and oxidative stress of Graves' disease. Why did the author choose puerarin for research? Please explain the reason.
Reply 4: Thank you for your insightful questions. Our research has also been fueled by extensive literature on the therapeutic potential of various drugs and genes in modulating inflammation and oxidative stress to enhance GD. Our selection of puerarin is based on previous research screening that suggests its potential as a protective factor against environmental disturbances in children with GD (references for details: 2). It has demonstrated clear therapeutic effects in various anti-inflammatory, antioxidant, and immune-related diseases. In addition, it is readily available and more cost-effective in the southern region of China. However, its potential as an environmental protective factor in preventing GD has not been previously investigated. Therefore, this study aims to establish a theoretical foundation for GD prevention and explore the viability of utilizing puerarin as a treatment option for GD.
Changes in the text: We have modified our text as advised (see page 15, line 266-271, see page 16, line 281-283)

Comment 5: This study uses puerarin alone. If puerarin is combined with Western medicine, how effective is it? It is suggested to add comparative analysis.
Reply 5: The valuable comments you provided are greatly appreciated. They have enlightened us on ideas for our further study. This study builds upon previous research on blood metabolomics in children with Graves' disease (GD) and normal children, identifying environmental disruptors as a potential protective factor against the onset and progression of GD. Our focus is on preventing GD, and our next step will be to investigate whether puerarin can be used as a therapeutic drug for GD treatment, either alone or in combination with Western medicine.
Changes in the text: We have modified our text as advised (see page 18, line 326-328)

Comment 6: The introduction part of this paper is not comprehensive enough, and the similar papers have not been cited, such as "Characterization of Graves' disease development after partial thyroidectomy for thyroid cancer, Transl Cancer Res, PMID: 35116624". It is recommended to quote the article.

Reply 6: Thank you for your valuable suggestion. The quotation from this article has been cited into the introduction section (4).
Changes in the text: We have modified our text as advised (see page 4, line 46)

Comment 7: The research progress of Chinese medicine in the treatment of Graves' disease should be added to the discussion.
Reply 7: Thank you for your valuable feedback. We have incorporated the latest research on Chinese medicine's efficacy in treating Graves' disease into our discussion section.
Changes in the text: We have modified our text as advised (see page 14-15, line 256-266)

Reviewer B

Comment 1: Figures
ALL abbreviations used in each figure or figure description should be defined in figure legend, please check and provide.
Reply 1: Thank you for your advices. We have added the descriptions for the abbreviations in each figure legend.
Changes in the text: We have modified our text as advised (see page 29, line 517-518, see page 30, line 526-528, see page 31, line 537-539, see page 32, line 545-547,)

Comment 2: Figure 2
Please a summarized legend for figure 2, followed by legends for each part.
Reply 2: Thank you for your valuable advices. We have summarized legend for figure 2.
Changes in the text: We have modified our text as advised (see page 29, line 515-523).

Comment 3: Figure 3
Please a summarized legend for figure 3, followed by legends for each part.
Reply 3: Thank you for your valuable advices. We have summarized legend for figure 3.
Changes in the text: We have modified our text as advised (see page 30-31, line 525-533).

Comment 4: Figure 5
There's no PI3K in the figure, please check.
Reply 4: Thank you for your advices. We have rephrased legend for figure 5.

Changes in the text:We have modified our text as advised (see page 32, line 542-548).

Comment 5: Figure 5 supplement

Please confirm whether this file will be published together with your study as a supplement file.

Reply 5: Thank you for your questions. we confirm the publication of this file as a supplement to our study, if the magazine needs.