Reviewer #1:

This study can be accepted in the present format. It will be of interest to the clinicians and researchers and also help in developing newer drugs for prevention and treatment of diabetic nephropathy. Congratulations to these authors for an excellent study demonstrating the molecular mechanisms for development of nephropathy in diabetes and potential role of ICAnin the management of diabetic nephropathy.

Response: We greatly appreciate your positive feedback and are glad to hear you find our research valuable. Thank you for your time and effort in reviewing our manuscript.

Reviewer #2:

In this study, Su et al investigated the mechanism of Icariin to regulate apoptosis in high glucose (HG) -induced primary rat kidney cells (PRKs). Firstly, the authors identified miR-503 to be upregulated in diabetic nephropathy. Then they showed that Icariin treatment could repress miR-503. Next, they provided data to show that SIRT4 is a target of miR-503. In summary, they identified a Icariin/miR-503/SIRT4 in diabetic nephropathy. Here I have the following concerns about this study.

1. A major issue is that there were little data to demonstrate the role of miR-503 in the pathology of diabetic nephropathy. This should be a necessary part for this research. Authors only revealed the correlation between miR-503 level and diabetic nephropathy. The causative data between them is required. They need to prove that miR-503 contribute to diabetic nephropathy.

Response: Thank you for your suggestion. In the current manuscript, we indeed focused on the correlative relationship. While we agree that further investigations to delineate the causative role of miR-503 in diabetic nephropathy would be valuable, such studies would involve extensive in vitro and in vivo manipulations that are beyond the scope of the present work. However, we have added a discussion about this in the manuscript to emphasize the importance and potential direction of future studies.

2. ERS could not be only determined by the expression of CHOP. Other markers are needed.

Response: Thank you for your suggestion. We also analyzed the activation of GRP78 in our study. When unfolded or misfolded proteins accumulate in the endoplasmic reticulum, this triggers an endoplasmic reticulum stress response. At the onset of stress, GRP78 dissociates from the sensor, which initiates the endoplasmic reticulum stress response, and this leads to upregulation of CHOP expression. Both GRP78 and CHOP, which were analyzed in this study, are key markers of ERS.

3. The role of miR-503 on the expression of SIRT4 should be demonstrated in different cell lines.

Response: Thank you for your suggestion. We agree with you that studying the effect of miR-503 on SIRT4 expression in different cell lines could provide broader insights. However primary rat kidney cells as well as high glucose treatment, which we believe is more relevant to the in vivo diabetic nephropathy setting. We mentioned in the revised manuscript that studies in different cell lines are a direction and goal for future research.

4. If the authors can validate their results in vivo, that will be better.
Response: Thank you for your suggestion. We concur with you about the importance of in vivo validation. However, due to the complexity and duration of in vivo experiments, they are currently beyond the scope of our study. We have added a section in the discussion noting that future studies could explore these effects in vivo.

5. In fig 3E, the SIRT4 band seemed over-exposed. Please replace it with a less-exposed band.
Response: Thank you for your suggestion. We replaced the other SIRT4 band in fig 3E.
6. In fig 4C, authors need to mark "SIRT4 WT" and "SIRT4 Mut". In the manuscript and figure legend, this luciferase assay should be depicted in detail.
Response: Thank you for your suggestion. We have added the relevant markers in fig 4C.

7. The expression "HG induction" is confusing and not appropriate. Authors need to revise it.
Response: Thank you for your suggestion. Thank you for pointing this out. We have replaced the term "HG induction" with "HG treatment" throughout the manuscript.

Reviewer #3:
In this study, the authors have investigated the potential molecular mechanism by which Icariin (ICA) prevents high glucose (HG)-induced endoplasmic reticulum (ER) stress-dependent apoptosis by regulating miR-503/SIRT4 axis in primary rat kidney (PRK) cells. This study is potentially interesting and innovative, but the reviewer has several concerns that the authors should address before considering its publication in this journal.
1. The title should be changed since currently it is a conclusive; however, this study was only based on cultured cells, therefore, there was no evidence to support these findings in the cultured cells exposed to only high levels of glucose for 24 or 48 hr with and without ICA can be recaptured in diabetic rats, no evidence whether ICA treatment also regulate the miR-503 and ER stress as seen in the vitro study. Therefore, the authors should not conclude

“miR-503 promotes the progression of diabetic nephropathy ……”
Response: Thank you for your suggestion. The title has been revised to "Investigation of a potential role for microRNA-503 in Icariin-mediated prevention of high glucose-induced endoplasmic reticulum stress"

2. Abstract: (1) Lacking miR-503 and SIRT4 information in AIM, which two are very important component in this vitro study; (2) Lacking animal model and HG experimental information; (3) Conclusions need to be revised based on what the authors have done and seen.
Response: Thank you for your suggestion.
(1) We added information about miR-503 and SIRT4 to the AIM.
(2) We added the description of HG-treated PRKs as an in vitro DN model in METHODS.
(3) We have made extensive revisions throughout the Discussion section.

3. Keywords should include one “Kidney damage” or “Diabetic kidney injury”
Response: Thank you for your suggestion. We have added "Kidney damage" to the list of keywords.

4. Several comments regarding the Introduction, Methods, Results are directly provided in the manuscript. Generally these include (1) need clearly presenting how innovative of this study; (2) clearly presenting the model information for both in vitro and in vivo; lacking information for how many times of the vitro experiments were repeated and whether the cells for each experiments were come from different isolations from the rats (3) Since you do not have DN evidence (renal dysfunction and remodeled kidney pathology), DN should be removed from figures of results; 4) Discussion needs focusing on what you found, do not imply its directly to DN.
Response: Thank you for the comment.
4.1 We have revised the introduction and discussion sections to further emphasize the novelty and significance of our study. The proposed changes include the introduction of the Icariin/miR-503/SIRT4 axis in the pathogenesis of HG-induced PRKs, which to our knowledge has not been previously reported. Our study thus provides a novel potential therapeutic target for HG-induced PRKs, which may have a significant impact on patient care in the future.
4.2. We added more descriptions of the in vivo and in vitro models in the methods section. In the "Statistical analysis" section, "The data were expressed as mean ± standard deviation (SD) of triplicate measurements." in the "Statistical analysis" section, we specified three replicates.
4.3. We changed the DN in the outcome graph to Hyperglycemic.
4.4. We have made extensive revisions throughout the Discussion section.