Dear Editor,

We truly appreciate you assigning such qualified reviewers to our manuscript. His/Her efforts and insights were a tremendous help to us during this revision. We would like to express our gratitude to you, the editorial team, and the reviewer whose valuable comments on this paper have significantly improved its quality. We have addressed all the comments in the new version of the paper and provided a point-by-point response to the reviewer's comments. These changes will not influence the content and framework of the paper. The reviewer comments are laid out below in italicized font and specific concerns have been numbered. Our response is given in normal font and and highlighted the revised/added contents with yellow color in the revised manuscript.

We hope that the changes we have made resolve all your concerns about the article. Once again, our thanks for your corrections and comments, and hope that you find the paper acceptable this time.

Please note that the modifications and the new subsection added have been highlighted in red font in "Manuscript ID number: 88216, Title of paper: Atorvastatin ameliorated myocardial fibrosis in db/db mice by inhibiting oxidative stress and modulating macrophage polarization".

Reviewer 1:

Comment 1: *The language quality is quite poor with a huge lot of grammar and syntax errors which need corrections with the help of a language editing/ medical editing service.*

Answer 1: Thank you for underlining this deficiency. We have sent our revised manuscript to a professional English language editing company to polish the manuscript further. Spelling and grammar issues have been revised. We will pay more attention to grammar in the future. We provide a language certificate along with the manuscript.

Comment 2: *Abstract: has several abbreviations without appropriate expansion of them initially which would make understanding difficult.*

Answer 2: Thank you for your reminder. We have added the full names of molecules and experimental methods in the abstract. This will make it easier for readers to understand. We have also added a list of abbreviations at the end of the manuscript. The modifications and the new added contents have been highlighted in yellow color in the revised manuscript.

Comment 3: *Introduction: - Authors should have elaborated how inflammatory cytokines (such as TGF-β1, TNF-a and IL-1β) impact on cardiac fibrosis with appropriate references as authors elaborates them in the other sections.*

Answer 3: Thank you for your nice suggestion. We read some relevant papers and elaborated how TGF-β1, TNF-α and IL-1β impact on cardiac fibrosis. These inflammatory cytokines are important players in cardiac inflammatory and fibrotic pathways, such as the TGF-β1/Smads pathway, the MAPK pathway, and the NLRP3/IL-1β pathway. The modifications and the new added contents have been highlighted in yellow color in the introduction section.

Comment 4: *Introduction: - Metformin is not a "hypoglycemic drug" (though can bring down glucose levels in diabetics) as authors propose.*

Answer 4: Thank you for your valuable comment. We couldn't agree more with your comments. We're not describing metformin very well. It is true that metformin is not a "hypoglycemic drug". As a first-line drug for glycemic control, the mechanism of metformin is to affect the ability of hepatocytes, myocytes, and adipose cells to process glucose, so that metformin not only inhibits glucose production in the liver, but also increases insulin sensitivity in peripheral tissues. It helps patients to fully utilize endogenous insulin, and lowers fasting and postprandial glucose. We have corrected the description of metformin in the first line of the last paragraph of the introduction section.

And we've added a paper describing how metformin can be used as a positive control drug in diabetic cardiomyopathy. (Carvacrol Attenuates Diabetic Cardiomyopathy by Modulating the PI3K/AKT/GLUT4 Pathway in Diabetic Mice. doi: 10.3389/fphar.2019.00998. PMID: 31572181; PMCID: PMC6751321.). The modifications and the new added contents have been highlighted in yellow color in the introduction section.

Comment 5: *Methods: - Why only those mice with random glucose levels ≥ 16.7 mmol/L were considered diabetic?*

Answer 5: We think this is a nice comment. Random blood glucose ≥ 16.7 mmol/L has been reported in the following articles in methods section as the criterion for mice diabetes. The criteria we used based on these articles:

1. Evaluation of the efficacy of *Abelmoschus manihot* (L.) on diabetic nephropathy by analyzing biomarkers in the glomeruli and proximal and distal convoluted tubules of the kidneys. doi: 10.3389/fphar.2023.1215996. PMID: 37587982; PMCID: PMC10427220.
2. Ulinastatin attenuates diabetes-induced cardiac dysfunction by the inhibition of inflammation and apoptosis. doi: 10.3892/etm.2017.4824. Epub 2017 Jul 19. PMID: 28962186; PMCID: PMC5609313.
3. Kirenol alleviates diabetic nephropathy via regulating TGF-β/Smads and the NF-κB signal pathway. doi: 10.1080/13880209.2022.2112239. PMID: 36073930; PMCID: PMC9467559.
4. Rspo3 regulates the abnormal differentiation of small intestinal epithelial cells in diabetic state. doi: 10.1186/s13287-021-02385-8. PMID: 34099046; PMCID: PMC8186182.

We have added a reference to the manuscript in the animals and treatment section. The modifications and the new added contents have been highlighted in yellow color in the methods section.

Comment 6: *Results: - It would be interesting to know if any of the experimental mice didn't respond to statin and metformin treatment without the changes authors identified.*

Answer 6: Thanks for your nice comment. In the present study, we found many interesting results, but the limitation of this study is the small sample size of mice used for the study, and mice that did not show a response to statins and metformin have not yet been observed. Therefore, it is also important to expand the sample size or come further at the cellular level to explore the effect of atorvastatin on macrophage polarization and cardiac fibrosis.

Comment 7: *Results: - The ALT and AST appears high among metformin arm, please explain.*

Answer 7: Thanks for your nice comment. Due to the different metabolic characteristics between species, ALT and AST in the blood of normal control mice are higher than that of humans. ALT and AST were no statistically differences between the four groups, regardless of whether they were diabetic mice or not, or whether they were with or without added drug intervention.

Comment 8: *Results: - There is no mention about Troponins in the results section. Why?*

Answer 8: Thank you for your question. In our manuscript, we mentioned troponin in "Effects of atorvastatin on cardiac function and structure in db/db mice" of the results section, along with LDH and CK-MB.

Comment 9: *Results: - Figure 4 wrongly mentions TNF-β1.*

Answer 9: Thank you for your careful checks. We were really sorry for our careless mistakes. We have corrected the mistake in Figure 4 (D).

Comment 10: *Discussion: - This section should have explored any human studies*

investigating the benefit of statins & metformin in ameliorating the chance of DCM.

Answer 10: Thank you for your valuable suggestion. According to some clinical trials, statins were initially used to lower blood lipids, however, in addition to their lipid-lowering effects, statins are involved in the regulation of the inflammatory response and play an important role in cardiovascular protection, quickly becoming a pillar in the prevention and treatment of cardiovascular disease. Metformin also showed partial cardiovascular protection. We added these important clinical trials to support and enrich our paper in the discussion section as you suggested. The modifications and the new added contents in discussion section have been highlighted in yellow color in the discussion section.

Comment 11: *References: - Needs more as mentioned already.*

Answer 11: According to your nice suggestions, we have read and cited some excellent papers to support our findings. We highlighted the added contents with yellow color in the revised manuscript.

Finally, we sincerely thanks for your valuable feedback that we can use the feedback to improve the quality of our manuscript. Thank you again for your valuable time and effort in this study.

Reviewer 2:

Comment 1: *It would be better if the authors add a graphical abstract to the manuscript.*

Answer 1: Thank you for your valuable suggestion. According to your suggestion, we have prepared a graphical abstract to show systematically what we have studied. We believe it is more intuitive to show what we are studying when we used graphical abstract. The graphical abstract is located before figure 1.

Comment 2: *In the animals and treatment section, the duration of treatment, sampling method, sample size and method of sacrificing mice should be explained in more detail. Also, in this section, authors should use "mice were sacrificed" instead of "mice were killed".*

Answer 2:

Thank you for your honest advice. We have added details to the manuscript that we missed. We highlighted the added contents with yellow color in the animals and treatment section.

Sample size: All mice were housed in standard cages (5 mice/cage). The control group consisted of 5 C57 mice. 15 db/db mice were divided into three groups randomly using a random number method.

The duration of treatment: Atorvastatin and metformin were dissolved in sterilized water and administrated by gastric gavage once a day for 16 weeks.

Sampling method: At 8 weeks of mice, blood glucose was measured in whole blood collected from the tail vein by a portable glucometer (Accu-Chek Active, Roche Diagnostics Limited, Mannheim, Germany). At 24 weeks of mice, all mice were fasted for 12 hours. Blood samples were collected from the ophthalmic vein and were used to test blood biochemistry indexes. We quickly remove the heart through chest surgery after sacrificing of the mice.

Method of sacrificing: Mice were sacrificed by cervical dislocation after 4% chloral hydrate anesthesia (0.20mL/20g; ip), and all efforts to minimize suffering were exerted.

Comment 3: *It is important that authors add more details of primers in a Table, including: Tm, Product lengths, and ACCESSION.*

Answer 3: Thank you for your suggestion. We prepared a new table (Table 2) to present the primer information. Table 2 is located at the table section.

Comment 4: *Please the authors explain how many sections and fields were investigated in histological Evaluations?*

Answer 4: Thank you for your nice comment. Three sections and fields were investigated in histological evaluations in each group. We highlighted the added contents with yellow color in the histological section.

Comment 5: *I suggest authors add a list of abbreviations at the end of the manuscript.*

Answer 5: Thank you for underlining this deficiency. We added a list of abbreviations at the end of the manuscript, below the table section.

Once again thanks for your courtesy in examining our manuscript. Your comments have enriched our paper and helped to improve the quality of our paper.

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With kind regards,

Sincerely yours,

Hong Zhou

28-Sep-2023