

Peer Review File

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Review comments-Reviewer A

Introduction:

Well-structured with a clear introduction, detailed information on the complications of erectile dysfunction in diabetes mellitus, and a shift towards the focus of the study on the potential therapeutic effects of NO₂-OA. Subsections discussing the mechanisms of DMED, pathways involved, and the introduction of NO₂-OA enhance the overall structure. Rationale for the study is well-supported by citing relevant statistics on the incidence of ED in DM patients and emphasizing the need for a deeper understanding of the mechanisms to develop new interventions.

Methods:

Provides a detailed and clear account of the procedures employed in the study. Information is well-organized, and the specifics of the interventions, such as the dosage of NO₂-OA and the vehicle used, are clearly outlined. It contains sufficient details about the procedures, measurements, and materials used, which is crucial for the reproducibility of the study. The mention of an established method (APO) for evaluating erectile function and the source of NO₂-OA adds to the transparency of the study. The description of the techniques used, such as the Western blot, Confocal Immunofluorescent Assay, Real-time Quantitative PCR, and Masson's trichrome staining, is adequately detailed. Inclusion of specific antibodies and concentrations used enhances the technical clarity.

Results:

Well-organized and presents the findings in a clear and systematic manner. Statistical significance of the results is appropriately presented. However, it would be beneficial to include specific statistical values (p-values) in addition to the description of significance, providing a more quantitative understanding of the findings. Effectively communicates the outcomes of the study, providing a clear narrative supported by visual representations. Including specific statistical values would enhance the precision of the reported findings.

Discussion

The discussion presents a comprehensive exploration of the study's findings, elucidating the role of NO₂-OA in improving erectile function in streptozotocin-induced diabetic rats. The paragraph is well-structured, providing a logical flow from the introduction of the study's context to the interpretation of results and their implications.

Comment 1:

In the sentence "However, NO₂-OA significant improve the level of autophagy...", there seems to be a typographical error ("significant" instead of "significantly"). While the findings are promising, exercise caution in the interpretation of results. Use language that reflects the limitations of the study and the need for further research to establish causation.

Reply 1: We thank the reviewer for the nice summary of our study firstly, and thank the comment and suggestion. We have modified our text as advised

Changes in the text: See Page 14, lines 261.

Review comments-Reviewer B

The manuscript titled "Nitrooleic acid (NO₂-OA) improves erectile dysfunction in a diabetic rat model by regulating fibrosis, inflammation, and autophagy" presents a report on the effects of nitrooleic acid (NO₂-OA) on diabetic rats. Extensive research on models of diabetic erectile dysfunction (DMED). The study was methodologically sound, with clear explanations of experimental design, sample size, and statistical analysis. Overall, this article contributes to the understanding of DMED and provides promising insights into future therapeutic approaches. However, there are several areas of potential improvement that should be fixed before accepting a release:

Comment 1: Although the methods section is generally clear, a more detailed description of certain procedures could improve reproducibility. For example, it would be helpful to detail the exact procedures for APO testing and cavernous nerve electrical stimulation.

Reply1: We have modified our text as advised.

Changes in the text: See Page 6, lines 103-106.

Comment 2: The Discussion section will be strengthened by more explicitly acknowledging the limitations of the study, including any potential bias, the generalizability of the findings, and the relevance of the rat model to the human condition. Authors should consider discussing any limitations and potential for human applications to further enhance the impact of the manuscript.

Reply 2: We have modified our text as advised.

Changes in the text: See Page 15, lines 272-276.

Comment 3: Improving the clarity and quality of figures and tables (>300dpi) to ensure that they effectively illustrate and support the text content will improve the overall readability of the manuscript.

Reply 3: We have improved the clarity and quality of figures and tables (>300dpi) as advised.

Changes in the text: -

Comment 4: Although the manuscript is generally well written, thorough proofreading to correct minor grammatical and typographical errors would enhance its professionalism.

Reply 4: We have modified our text as advised.

Changes in the text: -

Review comments-Reviewer C

This manuscript describes the effects of Nitro-oleic (NO₂-OA) on erectile dysfunction model in rat model of diabetes mellitus. The authors performed in vivo, morphological and biochemistry analysis in order to investigate the NO₂-OA effects. The authors concluded that NO₂-OA enhance the erectile function by inhibiting inflammation and fibrosis along with activating autophagy. The results are good, however there are some minor points that need to be addressed before the recommendation.

Comment 1: Why the authors investigated only one frequency-response on in vivo erectile function? Do you think that evaluate only one-point in vivo assessment it is a good experimental design? Why the authors did not perform a frequency- response curve?

Reply 1: We employed this method consisting of electrical stimulation of the cavernous nerve to assess erectile dysfunction in rats as it serves as a relatively easy, valid and reliable procedure that can provide an objective and accurate evaluation of the degree of penile erection. Moreover, we have previous experience with this method and, it represents a method that has been used in a number of studies worldwide which enables us to relate and compare our results with those of these other studies. It has been reported in the literature that 1.5V-7.5V provides suitable levels of voltages to test for erectile responses, while voltages exceeding 10V can produce burns. Therefore, with the use of an intermediate voltage of 5V we were able to achieve a safe, reliable and effective level of stimulation to activate the cavernous nerve in this experiment.

Although no definitive agreement currently exists within the academic community regarding the optimal stimulation parameters to test for ED, for the reasons as described above we believe the protocol used in our study provided the most effective means of assessing erectile function in these rats.

Changes in the text: -

Comment 2: The NO₂-OA improve the effects of any other drug used in the pharmacotherapy of erectile dysfunction such as iPDE V? Please add a statement on limitation section

Reply 2: We have modified our text as advised.

Changes in the text: See Page 15, lines 272-276.

Comment 3: Why the effects of only one dose of NO₂-OA was investigated? Pleased add a statement on limitation section

Reply 3: We have modified our text as advised.

Changes in the text: See Page 15, lines 272-276.