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Peer Review Report for "Allicin Alleviates Aortic Dissection Progression via p38/MAPK/NF-κB Signaling Pathways"

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Review Timeline:

Submit Date:	13 Aug 2025
Revised Date:	4 Oct 2025
Accepted Date:	5 Oct 2025

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

Esmail Mohammadian : Reviewer

15 Aug 2025

Dear Editor

Have a nice day.

The author investigated "Allicin alleviates aortic dissection progression via p38/MAPK/NF-κB signaling pathways".

I would suggest reconsidering the manuscript for publication only after the following major revision.

1. Please revise the manuscript for lingual quality and typo mistakes.
2. Given the title, the background section should be written better.
3. Titles and subtitles should be corrected.
4. Quantitative results should be mentioned in the abstract along with qualitative

results.

5. Please provide valid references for the method section.
6. All results should be reported with SD.
7. The article needs a complete revision.
8. A more complete conclusion section should be written.

REFEREE : Reviewer

16 Aug 2025

This study demonstrates that allicin effectively attenuates aortic dissection (AD) progression in a BAPN-induced mouse model by reducing mortality, aortic dilation, extracellular matrix degradation, and vascular smooth muscle cell (VSMC) phenotypic switching, while also suppressing oxidative stress and inflammation through inhibition of the p38/MAPK and NF- κ B signaling pathways—highlighting its potential therapeutic value; however, these promising findings require revision and strengthening through clarification of dosing relevance, validation of pathway specificity, inclusion of cytotoxicity controls, correction of data inconsistencies, and more rigorous methodological reporting to support robust conclusions.

1. The study uses oral administration of allicin at 14 mg/kg/day in mice. However, allicin is known to be highly unstable in gastric conditions and has poor bioavailability. Could the authors provide evidence (e.g., plasma concentration data, stability studies, or prior pharmacokinetic references) supporting that the orally administered dose results in biologically active concentrations reaching the aortic tissue?
2. While the data suggest inhibition of p38/MAPK and NF- κ B signaling, no direct mechanistic validation (e.g., use of pathway-specific inhibitors, siRNA knockdown, or phospho-protein time-course analysis) was performed. How can the authors rule out off-target effects or confirm that these pathways are directly responsible for the observed phenotypic changes rather than being secondary consequences?
3. To establish causality in the proposed mechanism, would the authors agree that overactivation of p38 or NF- κ B (e.g., via constitutively active mutants or agonists) should reverse the protective effects of allicin? Without such rescue experiments, how confident can we be that this pathway is the central mediator?
4. In cell experiments, allicin was used at 150–300 μ M, whereas the in vivo dose corresponds to much lower estimated plasma levels. What is the rationale for using such high in vitro concentrations, and how do these translate to physiological relevance? Could cytotoxicity at these doses confound interpretation of anti-migratory or anti-inflammatory effects?
5. The CCK-8 assay was mentioned in the methods but no data on cell viability under allicin treatment (especially at 150–300 μ M) were presented. Could the observed reduction in migration or cytokine production be due to reduced cell viability rather than specific modulation of VSMC phenotype?
6. In the results (line 123–124), it states that allicin reduced mortality from 45% to 35%, yet the survival rate increased from 50% to 70%. This appears contradictory. Can the authors clarify this discrepancy and ensure consistency in reporting mortality vs. survival rates across the text and figure?

7. Each group had n = 20 mice, but no power calculation was mentioned. Given the observed effect sizes (e.g., 70% vs. 40% AD incidence), was the sample size sufficient to detect statistically significant differences with adequate power? Could the study be underpowered for some endpoints?
8. Were the investigators blinded during histological evaluation, image quantification, and outcome assessment? Was randomization truly concealed during group allocation? Lack of blinding could introduce bias, especially in subjective measurements like histopathology scoring.
9. The BAPN model inhibits lysyl oxidase and causes elastin fragmentation, which mimics some features of AD but lacks key triggers like hypertension or inflammation seen in human AD. How well does this model recapitulate the complexity of human aortic dissection, and what are the limitations in translating these findings to clinical settings?
10. Only male mice were used in this study. Given known sex differences in cardiovascular disease progression and drug response, how might the findings differ in females? Do the authors plan to validate these results in female animals?
11. The intervention lasted 28 days, starting concurrently with BAPN. Would allicin still be effective if administered after AD initiation? This is crucial for therapeutic relevance. Can the authors comment on whether allicin halts progression or merely prevents onset?
12. Several antibodies (e.g., anti-NF- κ B, anti-p38) were used in immunofluorescence, but no information is provided about whether they detect activated (phosphorylated) forms. Since the pathway activity depends on phosphorylation, were phospho-specific antibodies used to assess activation status?
13. DCFH-DA is prone to artifacts and non-specific oxidation. Was any antioxidant control or alternative ROS probe (e.g., DHE for superoxide) used to confirm the specificity of the fluorescence signal? How did the authors control for potential interference by allicin itself on the fluorescence readout?
14. The conclusion suggests allicin may be a promising therapeutic agent for AD. However, no human tissue, clinical correlation, or ex vivo human VSMC data were included. Given the gap between rodent models and human disease, should the conclusions be tempered to reflect the preliminary nature of these findings?

REFeree : Associate Editor

22 Aug 2025

The Ms needs some major revisions before suitable for publication in JJNPP

REFeree : EIC

22 Aug 2025

The manuscript has been examined by expert reviewers who have concluded that the work is of potential interest to the readership of JJNPP.

However, from their comments it is clear that major revision of this manuscript will be required before a decision can be made on its publication. I would be pleased to consider a revised manuscript that addresses the reviewers' concerns. Your revised

manuscript will be returned to one or more of the original reviewers for further consideration.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

To Reviewer 1:

Question 1: The study uses oral administration of allicin at 14 mg/kg/day in mice. However, allicin is known to be highly unstable in gastric conditions and has poor bioavailability. Could the authors provide evidence (e.g., plasma concentration data, stability studies, or prior pharmacokinetic references) supporting that the orally administered dose results in biologically active concentrations reaching the aortic tissue?

Response to Question 1: We acknowledge the limited oral bioavailability of allicin. However, based on previous literature where allicin has been used at the same or similar doses in cardiovascular models (DOI: 10.1016/j.phymed.2018.11.025, DOI: 10.18632/aging.203578), demonstrating efficacy and safety, and following dose conversion and preliminary experiments with multiple concentration gradients, we selected the dose of 14 mg/kg/day. Although we did not measure plasma concentrations in this study, the significant reduction in AD incidence and the improvement in histological and biochemical endpoints provide indirect evidence of its bioactivity. Future studies will include pharmacokinetic assessments to confirm tissue distribution.

Question 2: While the data suggests inhibition of p38/MAPK and NF- κ B signaling, no direct mechanistic validation (e.g., use of pathway-specific inhibitors, siRNA knockdown, or phospho-protein time-course analysis) was performed. How can the authors rule out off-target effects or confirm that these pathways are directly responsible for the observed phenotypic changes rather than being secondary consequences?

Response to Question 2: We agree that utilizing pathway-specific inhibitors or gene knockdown technology would provide more profound and definitive results. Our immunohistochemistry results offer preliminary evidence regarding the effect of allicin on the p38/MAPK and NF- κ B pathways. In the revised manuscript, we have toned down the causal language and emphasize that these findings suggest a potential mechanism, which will require further validation in future studies employing pathway-specific modulators.

Question 3: To establish causality in the proposed mechanism, would the authors agree that overactivation of p38 or NF- κ B (e.g., via constitutively active mutants or agonists) should reverse the protective effects of allicin? Without such rescue experiments, how confident can we be that this pathway is the central mediator?

Response to Question 3: We appreciate this insightful comment. We have revised the discussion to indicate that future studies will include gain-of-function experiments to establish causality.

Question 4: In cell experiments, allicin was used at 150–300 μ M, whereas the in vivo dose corresponds to much lower estimated plasma levels. What is the rationale for using such high in vitro concentrations, and how do these translate to physiological relevance? Could cytotoxicity at these doses confound interpretation of anti-migratory or anti-inflammatory effects?

Response to Question 4: We sincerely thank the reviewer for this keen observation. This was indeed an oversight in our writing process. For the cell experiments, based on previous

studies(DOI:10.3969/j.issn.1006-6233.2020.06.016;10.16368/j.issn.1674-8999.2022.04.152;10.7661/j.cjim.20180129.041), we initially conducted preliminary experiments with different concentration gradients. Based on these results, we ultimately selected an allicin concentration of 20 μ M for the formal experiments. We have corrected this in Methods section 2.3 of the manuscript. We apologize for any confusion caused and thank you for helping us improve the accuracy of our paper.

Question 5: The CCK-8 assay was mentioned in the methods but no data on cell viability under allicin treatment (especially at 150–300 μ M) were presented. Could the observed reduction in migration or cytokine production be due to reduced cell viability rather than specific modulation of VSMC phenotype?

Response to Question 5: Thank you for your question. Based on previous research (DOI: 10.3969/j.issn.1006-6233.2020.06.016, 10.16368/j.issn.1674-8999.2022.04.152), cell viability is not affected at allicin concentrations $\leq$ 60 μ M. Combined with the clarification in the previous question, our experiments utilized an allicin concentration of 20 μ M.

Question 6: In the results (line 123–124), it states that allicin reduced mortality from 45% to 35%, yet the survival rate increased from 50% to 70%. This appears contradictory. Can the authors clarify this discrepancy and ensure consistency in reporting mortality vs. survival rates across the text and figure?

Response to Question 6: Thank you for your meticulous observation and question. The discrepancy between our description and the figure was a typographical error, which has been corrected in the revised manuscript. Figure 1.A shows the AD induction rate in each group of mice, while Figure 1.B shows the survival curves. AD development is a dynamic process; in the early stages, the aorta generally does not rupture, posing a relatively minor threat to survival. However, when AD progresses to a certain extent, aortic rupture can occur, leading to extremely high mortality in mice without prompt intervention. Therefore, the AD incidence rate is not equivalent to the mortality rate, and surviving mice may not necessarily have completely healthy aortas.

Question 7: Each group had $n = 20$ mice, but no power calculation was mentioned. Given the observed effect sizes (e.g., 70% vs. 40% AD incidence), was the sample size sufficient to detect statistically significant differences with adequate power? Could the study be underpowered for some endpoints

Response to Question 7: Thank you for your question. Based on a previous research paper (DOI: 10.1161/CIRCULATIONAHA.121.056640), we selected $N=20$. The observed effect size (e.g., a 40% reduction in AD incidence) was statistically significant ($p < 0.05$).

Question 8: Were the investigators blinded during histological evaluation, image quantification, and outcome assessment? Was randomization truly concealed during group allocation? Lack of

blinding could introduce bias, especially in subjective measurements like histopathology scoring.

Response to Question 8: Thank you for your question. Histological evaluation and image analysis were performed by investigators blinded to the specific experimental groups.

Question 9: The BAPN model inhibits lysyl oxidase and causes elastin fragmentation, which mimics some features of AD but lacks key triggers like hypertension or inflammation seen in human AD. How well does this model recapitulate the complexity of human aortic dissection, and what are the limitations in translating these findings to clinical settings?

Response to Question 9: Thank you for your question. We acknowledge that the BAPN model does not fully replicate the complexity of human AD, such as the presence of hypertension or other pathologies. However, referring to previous studies (DOI: 10.1161/ATVBAHA.123.319281, DOI: 10.7150/ijbs.72528), the BAPN-induced AD model remains a widely used method with relatively high simulation fidelity and modeling success rate.

Question 10: Only male mice were used in this study. Given known sex differences in cardiovascular disease progression and drug response, how might the findings differ in females? Do the authors plan to validate these results in female animals?

Response to Question 10: Thank you for your question. We chose male mice for this study primarily for two reasons: 1) Clinically, the incidence of AD is higher in males than in females. 2) From an experimental animal perspective, hormone levels are more stable in male mice. We acknowledge that the effects of allicin may exhibit sex differences and plan to include female mice in future studies to evaluate potential sex-specific responses.

Question 11: The intervention lasted 28 days, starting concurrently with BAPN. Would allicin still be effective if administered after AD initiation? This is crucial for therapeutic relevance. Can the authors comment on whether allicin halts progression or merely prevents onset?

Response to Question 11: Thank you for your professional question. The current study primarily focuses on the preventive effects of allicin. Another potential therapeutic direction could target post-operative clinical AD patients. Since current clinical treatments for AD often involve resection of the dissected segment without fully ameliorating the underlying pathological environment (e.g., inflammation), AD can recur. Therefore, reducing the postoperative recurrence rate is a potential goal for drug therapy. We agree that therapeutic intervention after AD onset should prioritize surgical treatment supplemented by pharmacological therapy, and we plan to investigate the question of "whether allicin remains effective if administered after AD onset" in subsequent research.

Question 12: Several antibodies (e.g., anti-NF- κ B, anti-p38) were used in immunofluorescence, but no information is provided about whether they detect activated (phosphorylated) forms. Since the pathway activity depends on phosphorylation, were phospho-specific antibodies used to assess activation status?

Response to Question 12: Thank you for your valuable comment. You are absolutely correct; using phosphorylation-specific antibodies is a more direct method for assessing the activation status of the NF- κ B and p38 signaling pathways. We greatly appreciate you pointing this out. Due to limitations in technical resources, we were unable to supplement the revision with experiments using phospho-specific antibodies. We acknowledge this as a limitation of the present study and will prioritize this approach in future research to directly validate the activation of these pathways. Nevertheless, our current immunofluorescence data still provides some indirect evidence supporting pathway activation:

- For the NF- κ B pathway: A key hallmark of its activation is translocation from the cytoplasm to the nucleus. In our Figure 6, a significant shift of fluorescence signal from the cytoplasm to the nucleus can be observed in the model group, which is a strong indicator of canonical NF- κ B pathway activation.
- For the p38 pathway: Although the localization changes of total p38 protein are less pronounced, we observed upregulation of downstream inflammatory factors (TNF- α , IL-6), which is consistent with the phenotype of p38 pathway activation.

We believe that this indirect evidence, combined with other data in our manuscript, still supports our overall conclusions. Thank you again for helping us improve this paper.

Question 13: DCFH-DA is prone to artifacts and non-specific oxidation. Was any antioxidant control or alternative ROS probe (e.g., DHE for superoxide) used to confirm the specificity of the fluorescence signal? How did the authors control for potential interference by allicin itself on the fluorescence readout?

Response to Question 13: Thank you very much for these crucial and highly insightful comments. You are right; the DCFH-DA probe is indeed susceptible to non-specific oxidation and artifacts, and the potential interference of the drug itself on the fluorescence readout must be ruled out. We sincerely appreciate you identifying this shortcoming in our study. As the original cell lines are exhausted, we are unable to complete these critical control experiments for this revision, which we deeply regret. Nonetheless, based on the existing data, we aim to strengthen the reliability of our conclusions as much as possible: In our study, we observed that allicin treatment concurrently affected downstream pathways related to ROS, which is consistent with and supportive of the phenotype of changed ROS levels. We strictly followed standard protocols in our experiments, such as light-protected operation and consistent probe loading times, to minimize artifacts. Previous studies have confirmed that allicin can downregulate ROS production (DOI: 10.1186/s12906-016-1126-9, 10.3892/etm.2015.2708), which provides some corroborative support for our methodology.

Question 14: The conclusion suggests allicin may be a promising therapeutic agent for AD. However, no human tissue, clinical correlation, or ex vivo human VSMC data were included. Given the gap between rodent models and human disease, should the conclusions be tempered to reflect the preliminary nature of these findings?

Response to Question 14: Thank you for your question. We agree that the findings of this study are preliminary and derived from rodent models. We have adjusted the conclusions to

emphasize the potential mechanistic insights and the necessity for further validation in human tissues or clinical settings.

To Reviewer 2:

Question 1: Please revise the manuscript for lingual quality and typo mistakes.

Response to Question 1: We fully agree with the reviewers' comments and apologize for the language errors in our original manuscript. We have corrected all spelling and grammatical mistakes in the revised paper.

Question 2: Given the title, the background section should be written better.

Response to Question 2: Thank you for pointing this out. We have rewritten the Background section to better align with the title and the core content of our study.

Question 3: Titles and subtitles should be corrected.

Response to Question 3: We have reviewed and corrected the formatting of all heading levels to ensure clarity, consistency, and compliance with the journal's guidelines.

Question 4: Quantitative results should be mentioned in the abstract along with qualitative results.

Response to Question 4: We fully agree. The Results section in the abstract was indeed too general. We have now added key quantitative data to make it more compelling.

Question 5: Please provide valid references for the method section.

Response to Question 5: Thank you for pointing out this oversight. We have added appropriate references for the key experimental procedures and reagents described in the Methods section, including animal model establishment, compound dose selection, and cell-based experimental protocols.

Question 6: All results should be reported with SD.

Response to Question 6: We have reviewed the Results section and all figures/tables throughout the manuscript; every quantitative datum is now presented as mean $\pm$ SD.

Question 7: The article needs a complete revision.

Response to Question 7: We sincerely thank the reviewer for this comprehensive assessment. In response to all of your specific comments, including the present remark, we have undertaken a thorough and extensive revision of the manuscript. This has encompassed the language polishing, structural refinement, and reference supplementation noted above, and we believe the revised version is substantially improved.

Question 8: A more complete conclusion section should be written.

Response to Question 8: We agree that the original conclusion could be more in-depth. We have completely rewritten this section so that it not only summarizes the principal findings but also underscores the significance of the study, acknowledges its limitations, and outlines directions for future research.

OPEN PEER REVIEW

Revision (1)

Esmail Mohammadian : Reviewer

21 Sep 2025

Dear editor

Have a nice day.

The author studied Allicin alleviates aortic dissection progression via p38/MAPK/NF- κ B signaling pathways.

In my opinion, this manuscript cannot be considered for publication.

The main reason for this outcome is the following:

1. Responding to comments is not done well.

REFEREE : Reviewer

22 Sep 2025

While the paper is significantly strengthened, a few minor points could be addressed for absolute clarity.

Accept after Minor Revisions:

Bioavailability (Question 1): This remains the most significant conceptual gap. While they cite precedent and provide indirect evidence, the lack of PK data is a limitation.

However, they fully acknowledge this and promise future studies. For a first mechanistic study using a well-established dose from literature, this is acceptable.

Phospho-Specific Antibodies (Question 12): The inability to use phospho-antibodies is unfortunate. Their argument based on NF- κ B nuclear translocation is scientifically valid as an indicator of activation. While not as direct as phospho-staining, it is a widely accepted method. Again, they clearly state this as a limitation.

ROS Assay Controls (Question 13): The lack of controls for DCFH-DA is a methodological weakness. Their reliance on protocol adherence and correlative downstream data is reasonable but not ideal. This should be clearly stated as a limitation in the discussion.

REFEREE : Associate Editor

22 Sep 2025

Please provide a point-by-point response to each of the questions and suggestions

REFEREE : EIC

22 Sep 2025

Thank you for your careful revisions and thoughtful responses to the reviewers' comments on your manuscript. I appreciate the effort you have invested in strengthening the paper.

After reviewing both your revised manuscript and the reviewers' feedback, I am pleased to inform you that your manuscript is conditionally accepted pending minor revisions. The majority of concerns have been adequately addressed, and Reviewer 1 explicitly acknowledges the significant improvements made particularly regarding bioavailability, phospho-specific antibody limitations, and ROS assay interpretation while recommending acceptance with clear acknowledgment of methodological constraints.

However, Reviewer 2 has raised a concern that your point-by-point responses to the original critiques were not fully satisfactory or sufficiently detailed. While this does not invalidate the scientific merit of your work, which Reviewer 1 found compelling, it is important that all reviewer concerns are met with clear, thorough, and respectful replies to ensure transparency and scholarly rigor.

OPEN PEER REVIEW

Revision (2)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

To Reviewer 1:

Question 1: The study uses oral administration of allicin at 14 mg/kg/day in mice. However, allicin is known to be highly unstable in gastric conditions and has poor bioavailability. Could the authors provide evidence (e.g., plasma concentration data, stability studies, or prior pharmacokinetic references) supporting that the orally administered dose results in biologically active concentrations reaching the aortic tissue?

Response to Question 1: We sincerely thank the reviewer for raising this important point. We acknowledge that allicin has limited oral bioavailability due to its instability under gastric conditions. Our dosing regimen (14 mg/kg/day) was selected based on dose conversion, preliminary concentration-gradient experiments, and prior reports where similar oral doses demonstrated cardiovascular efficacy and safety (DOI: 10.1016/j.phymed.2018.11.025; DOI: 10.18632/aging.203578).

Importantly, in our model, oral allicin administration resulted in a significant reduction in AD incidence and improved histological and biochemical parameters, providing indirect evidence of its bioactivity. We agree that direct pharmacokinetic validation is necessary, and we plan to include plasma and aortic tissue distribution analyses of allicin-derived metabolites in future studies to better correlate pharmacokinetics with therapeutic efficacy.

Question 12: Several antibodies (e.g., anti-NF- κ B, anti-p38) were used in immunofluorescence, but no information is provided about whether they detect activated (phosphorylated) forms. Since the pathway activity depends on phosphorylation, were phospho-specific antibodies used to assess activation status?

Response to Question 12: Thank you very much for this insightful comment. You are absolutely correct that phosphorylation-specific antibodies provide a more direct and reliable means of assessing the activation status of the NF- κ B and p38 signaling pathways. We truly appreciate your suggestion. Due to limitations in our technical resources, we were unable to perform additional experiments with phospho-specific antibodies for this revision, and we acknowledge this as a limitation of the current study. We will make this a priority in future research to more directly validate pathway activation.

Nevertheless, we believe our present data provide supportive indirect evidence. For the NF- κ B pathway, a hallmark of activation is its nuclear translocation, and in Figure 6, we observed a clear shift of the fluorescence signal from the cytoplasm to the nucleus in the model group, consistent with canonical NF- κ B activation. For the p38 pathway, although changes in total protein localization are less pronounced, the observed upregulation of downstream inflammatory cytokines (TNF- α , IL-6) aligns with the expected phenotype of p38 activation.

Taken together, while indirect, these findings support our conclusion that allicin attenuates NF- κ B and p38 pathway activation. We are grateful for your constructive feedback, which will guide the refinement of our future work.

Question 13: DCFH-DA is prone to artifacts and non-specific oxidation. Was any antioxidant

control or alternative ROS probe (e.g., DHE for superoxide) used to confirm the specificity of the fluorescence signal? How did the authors control for potential interference by allicin itself on the fluorescence readout?

Response to Question 13: Thank you very much for this crucial and insightful comment. We fully acknowledge that the DCFH-DA probe is prone to non-specific oxidation and artifacts, and that potential interference from allicin itself must be considered. We sincerely appreciate you highlighting this limitation. Unfortunately, as the original cell lines are no longer available, we were unable to perform additional control experiments for this revision, which we deeply regret.

Nevertheless, we have taken several measures to strengthen the reliability of our findings. All experiments strictly followed standard protocols, including light-protected operation and consistent probe loading times, to minimize artifacts. Importantly, the observed changes in ROS levels were consistent with downstream alterations in oxidative stress-related pathways, providing supportive indirect evidence. Furthermore, previous studies have reported that allicin can reduce ROS production in similar cellular contexts (DOI: 10.1186/s12906-016-1126-9; DOI: 10.3892/etm.2015.2708), corroborating our observations.

In future work, we plan to incorporate more specific ROS probes (e.g., DHE or MitoSOX) and antioxidant controls to further confirm the specificity of ROS changes and to fully exclude potential interference by allicin itself. We sincerely appreciate this constructive suggestion, which will help us refine our methodology in subsequent studies.

OPEN PEER REVIEW

Revision (2)

REFEREE : EIC

5 Oct 2025

I am pleased to inform you that your manuscript has been accepted for publication in JJNPP.