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OPEN PEER REVIEW

Comparing the Antibacterial Effects of 660 nm Laser Therapy on *Staphylococcus aureus* Using Phycocyanin and Methylene Blue: An In-Vitro Study

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About this report

This open peer review (OPR) report shows the public comments made on this article during peer review, by the reviewers, the associate editor and the editor-in-chief, for every revision round, together with the authors' replies. Comments appear only with the consent of the people who wrote them. Reviewers who chose to sign their report are named; the others are shown as "Referee". Comments are edited for language only, and confidential remarks to the editors are not included.

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1 Original submission

2 comments · 27 Oct 2024 – 28 Oct 2024

Associate Editor

MAJOR REVISION 27 Oct 2024

Please revise the manuscript according to the reviewers' comments.

Editor-in-Chief

MAJOR REVISION 28 Oct 2024

Dear Authors
According to the reviewers' and associate editor's comments, your article needs major revision. When revising your manuscript, please consider all issues mentioned in the reviewers' comments carefully: please outline every change made in response to their comments and submit the required files.

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

“Peer Review”

Manuscript ID: 155074
Title: The antibacterial effects of photodynamic therapy on staphylococcus aureus using phycocyanin and methylene blue: A comparative in vitro study

Dear Dr. Ehsani,

We are grateful for the feedback from you and the reviewers and have incorporated the suggested changes. Here is a point-by-point response to the reviewers' comments:

Reviewer comments

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Reviewer 1:

I read your valuable paper and I have some questions about your study:

1. How can we use this method in eradication of Staphylococcus aureus infections in-vivo?

Response: Thank you for your valuable comment. This part has been added to the discussion section (lines 133-190).

2. Can PDT cause microbial resistance in these type of bacteria?

Response: Thank you for your comment. It is highly unlikely that PDT will cause microbial resistance in S. aureus or other types of bacteria. The related reasons have been added to the discussion section (lines 133-190).

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Reviewer 2:

1. The writing is somewhat unclear in a few areas. For example, the explanation of the groups and concentrations could be more concise in abstract. It is better to limit the concentration used in the abstract to the lowest and highest concentration.

Response: Thank you for your feedback. The method section of abstract has been revised based on your comment. (lines 10-13)

2. The Conflict of Interests section is very, very high.

Response: Thank you for your valuable comment. The conflict-of-interest section follows the journal's format as shown on their website.

3. What do the authors mean by defective cycle in line 33? be explained

Response: with many thanks, the explanation has been added to the manuscript. (lines 33-41)

4. Lines 31 to 33 have writing problems and need to be corrected

Response: Thank you for your comment. This part was revised. (lines 30-41)

5. For the first paragraph of the introduction, 5 references have been announced, it is better to place the reference for each sentence in the relevant section.

Response: with many thanks, the citations has been revised based on your comment. (lines 28-32)

6. It is better to discuss this matter in the introduction: the main mechanisms through which photodynamic therapy causes the death of bacteria.

Response: with many thanks, this part has been added to the introduction. (lines 46-50)

7. How the specific concentrations of phycocyanin and methylene blue were selected for this study, and were they based on prior research or preliminary results?

Response: Thank you for your comments. The concentrations of phycocyanin and methylene blue (MB) chosen for this study were determined based on prior research and preliminary findings. Previous studies have demonstrated varying levels of effectiveness for both phycocyanin and MB as photosensitizers in photodynamic therapy (PDT) at different concentrations, which guided the selection of the concentration range. Relevant citations have been added. (line 98)

8. Why was an alpha error of 1% chosen for this experiment?

Response: with many thanks, by using a stricter alpha level of 1%, we try to reduce the likelihood of a Type I error, ensuring that any statistically significant results are more robust and less likely to be due to random chance. The reason has been added to the method section. (lines 66-68)

9. Add a brief explanation of why MB is more effective than phycocyanin at reducing colony numbers, particularly at lower concentrations.

Response: with many thanks, this part has been added to the discussion section. (lines 150-158)

10. Figures and tables are written on page 13 if there is only one table

Response: with many thanks, the title has been revised.

11. The number of references is too much compared to the content of the article. Some paragraphs have 3 to 4 references, which is not necessary. It is better to use newer references and reduce the number of references.

Response: Thank you for your comment. The references were revised based on your comment.

12. The text highlights conflicting findings regarding phycocyanin's antibacterial effects, but does not delve into why these discrepancies may exist. For example, environmental factors such as pH, bacterial growth conditions, or experimental design differences might contribute to varying outcomes. Offering a hypothesis on these conflicting results could strengthen the discussion.

Response: Thank you for your comment. The discussion section has been revised based on your comment (lines 133-190).

13. The text mentions studies showing MB’s higher efficiency at producing reactive oxygen species (ROS) in the presence of laser light but does not compare the mechanisms of ROS production between MB and phycocyanin.

Response: Thank you for your comment. The discussion section has been revised based on your comment (lines 133-190).

14. In the table there is a column named Number of mini-plates this column seems unnecessary and should be removed.

Response: Thank you for your comment. The column has been removed.

15. Numbers of colonies column in the table of numbers is wrongly typed and should be corrected.

Response: Thank you for your comment. The table has been revised.

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Associate Editor's Comments:

Associate Editor 1:

Please revise the manuscript according to reviewers' comments.

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Reviewer Assistant Comments:

These comments were generated by AI based on your manuscript. Please review them and consider incorporating the suggestions to enhance your article.

Based on the provided text regarding the study on the antibacterial effects of photodynamic therapy on Staphylococcus aureus using phycocyanin and methylene blue, I will apply the relevant checklists to ensure comprehensive coverage of the study's reporting standards.

Checklist Application

1. **Study Type**: This study appears to be an in vitro experimental study comparing the effects of two different photosensitizers (phycocyanin and methylene blue) under photodynamic therapy on bacterial colonies.

2. **Relevant Checklists**:

- **CONSORT Checklist**: Although this study is not a randomized trial, it may still benefit from some elements of the CONSORT guidelines, particularly regarding the methods and results sections.

- **STROBE Checklist**: Since this is an observational study, the STROBE checklist for cohort studies may be applicable, particularly for describing the study design and methods.

- **STARD Checklist**: If the study aims to evaluate the diagnostic accuracy of the photodynamic therapy as a treatment modality, elements from the STARD checklist may also be relevant.

Key Points from the Checklists

CONSORT Checklist

- **Title and Abstract**: The title should clearly indicate the study design and main findings.

Response: The title has been changed.

- **Background and Objectives**: The background should provide a rationale for the study, and specific objectives should be stated.

Response: The objective has been revised.

- **Methods**: The study design, setting, eligibility criteria, and statistical methods should be detailed.

Response: This is an experimental in-vitro study. The eligibility criteria section has been added and the statistical analysis has been clarified in the method section.

STROBE Checklist

- **Study Design**: The design should be clearly stated, including the setting and relevant dates.

Response: This is an experimental in-vitro study.

- **Eligibility Criteria**: Clear criteria for inclusion and exclusion of samples should be provided.

Response: The eligibility criteria section has been added to the method section.

- **Statistical Methods**: All statistical methods used for analysis should be described, including how confounding factors were controlled.

Response: All confounding factors were discussed in the limitation section of the discussion.

STARD Checklist

- **Participants**: The eligibility criteria for the bacterial strains used should be specified.

Response: The eligibility criteria section has been added to the method section.

- **Test Methods**: Detailed descriptions of the photodynamic therapy protocols and the measurements of bacterial colony counts should be included.

Response: It has been added to the method.

- **Results**: The results should present estimates of the effects of the treatments, including confidence intervals.

Response: It has been added to the result section.

Recommendations for Improvement

- **Detailed Methods**: Include more detailed descriptions of the methods used, such as how the bacterial colonies were prepared and the specific conditions under which the photodynamic therapy was applied.

Response: Method has been revised.

- **Statistical Analysis**: Clarify the statistical methods used for analyzing the data, including any adjustments for confounding variables.

Response: The method section has been revised.

- **Flow Diagram**: Consider including a flow diagram to illustrate the experimental process and the number of samples at each stage.

Response: A flow chart diagram has been designed and added to the manuscript.

Conclusion

The study provides valuable insights into the effectiveness of photodynamic therapy using different photosensitizers against S. aureus. However, enhancing the clarity and detail in the methods and results sections will strengthen the reporting and allow for better replication and understanding of the findings.

Response: with many thanks, the manuscript has been revised based on the comments.

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Best regards

Dr. S L

Assistant professor in Oral Medicine Department, Tehran Medical Sciences, Islamic Azad University, Tehran, Iran

2 Revision 1

2 comments · 11 Nov 2024 – 12 Nov 2024

Associate Editor

ACCEPT 11 Nov 2024

Thank you very much for your reply and for the corrections.

Editor-in-Chief

ACCEPT 12 Nov 2024

Dear Authors
We are pleased to inform you that your article is accepted for publication in the MEJRHS. We hope to receive other suitable articles from your group.