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OPEN PEER REVIEW

Curcumin-Etoposide Synergy: Unveiling the Molecular Mechanisms of Enhanced Apoptosis and Chemoresistance Attenuation in Breast Cancer

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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

1 comment · 15 Aug 2024

Associate Editor

MAJOR REVISION 15 Aug 2024

Dear authors,
Please see below the comments made by the reviewers on your submission. In conclusion, my decision is major revision. I ask you to please read the reviewers' comments carefully, respond to them point by point, and improve your manuscript accordingly.

With kind regards,
Associate editor

Review for IJPR- 150978
Reviewer 1:
Please note and ensure that the provided comments are carefully considered and that any necessary corrections are made to the relevant sections of the manuscript.
Background
1. The background section should offer a more comprehensive overview of the topic, encompassing pertinent studies and underscoring the existing knowledge gaps. This will serve to emphasize the significance and novelty of the research (in the last paragraph).
Material & Methods
2. Within the methods section 3.3, it is imperative to incorporate the wavelength and provide scientifically validated references when discussing the cell migration assay.
3. In section 3.4, consider amending the title to "Cell Migration Assay."
4. Subsequent to the mention of "YEKTA TAJHIZ" in section 3.5, it is essential to include the city and country, and specify the lot number of the manufacturer. Additionally, the working method should be elucidated further or supported by pertinent references.
5. Part 3.9 necessitates a more detailed account of the working method. Additionally, it is advisable to specify the type of kit used in this section.
Results
6. Regarding the results section, a revision is required for the initial sentence in section 4.2, line 171, to ensure accuracy and clarity.
7. When denoting significant increases or decreases in the results section, it is important to include the specific p-values in the text, as opposed to only representing them with asterisks in the captions of the graphs.
8. Enhancements in the quality and resolution are needed for the graphs and shapes, particularly in Figure 1, to meet the requisite standards of clarity and legibility.
9. In Figure 3, detailing the magnification levels in parts A and B will facilitate improved interpretation and comparison of the results.
Discussion
10. It is recommended to expand the discussion section by integrating relevant literature for comparisons, outlining the implications of the study findings, addressing potential limitations, and proposing avenues for future research.
Conclusion
11. The conclusion should be succinct yet impactful, encapsulating the significant outcomes of the study and emphasizing the relevance and applications of the research findings to the scientific community.
References
12. In the discussion section, certain references should be substituted with more pertinent and current

sources (particularly within the last 5 years).
Reviewer 2:
The study's lack of novelty makes it unfit for publishing in its current state. The research specifically focuses on subjects that have been thoroughly discussed in current literature.

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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.

Dear Editor-in-Chief

Thank you for evaluating our manuscript. We would also like to thank the reviewers very much for their additional feedback and suggestions. We are now re-presenting a significantly revised version of the original manuscript, in which we respond in detail to the reviewers' criticism. We responded to each reviewer's concerns point by point. We hope that our answers are satisfactory to you and that the manuscript is now approved for publication.

Reviewer 1:

Please note and ensure that the provided comments are carefully considered and that any necessary corrections are made to the relevant sections of the manuscript.

Background

1. The background section should offer a more comprehensive overview of the topic, encompassing pertinent studies and underscoring the existing knowledge gaps. This will serve to emphasize the significance and novelty of the research (in the last paragraph).

Dear Reviewer,

Thank you for your insightful feedback on the background section of our manuscript. We have taken your suggestion to heart and added a paragraph at the end of the background to give a more comprehensive overview of the topic. This new paragraph includes relevant studies and highlights the existing knowledge gaps, highlighting the importance and novelty of our research. We believe this addition will improve the clarity and depth of the background section and provide a stronger foundation for the research presented in our manuscript. Thank you again for your valuable contribution.

Material & Methods

2. Within the methods section 3.3, it is imperative to incorporate the wavelength and provide scientifically validated references when discussing the cell migration assay.

Dear Reviewer,

Thank you for your feedback regarding the cell migration assay described in our manuscript. We would like to clarify that the wound-healing assay we employed to evaluate cell migration does not involve the use of wavelength, as it is a visual and morphological assessment of cell movement over time. The assay primarily relies on the creation of a "wound" in a confluent cell monolayer and the subsequent measurement of the rate at which the cells migrate to close this wound.

The protocol for the wound-healing assay, as described in our manuscript, involves the following steps:

1. Cell Seeding: MCF-7 and MDA-MB-231 cells were seeded in 6-well plates and allowed to reach greater than 90% confluency.

2. Wound Creation: A 5 mL pipet tip was used to create a "wound" in the cell layer.

3. Cell Washing: The cells were washed with PBS to remove cell debris and non-attached cells.

4. Treatment: The cells were treated with curcumin and etoposide, respectively.

5. Imaging: Images were taken at 0, 24, and 48 hours of incubation with QC and QC-SLN.

6. Analysis: The distance of cell migration was calculated using the National Institutes of Health (NIH) Image J software, using the formula: The rate of migration = [(T0 - Th) / T0] * 100.

We have not used any specific wavelength in this assay, as it is a standard method for assessing cell migration based on visual inspection and measurement of the wound closure over time. The use of Image J software is a widely accepted and scientifically validated method for quantifying cell migration in wound-healing assays.

To ensure the scientific validity of our methods, we have cited relevant references that support the use of the wound-healing assay and the Image J software for quantifying cell migration. We believe that these references will provide the necessary context and validation for our approach.Thank you once again for your valuable feedback. We are committed to ensuring the accuracy and clarity of our methods.

3. In section 3.4, consider amending the title to "Cell Migration Assay."

Corrections were made.

4. Subsequent to the mention of "YEKTA TAJHIZ" in section 3.5, it is essential to include the city and country, and specify the lot number of the manufacturer. Additionally, the working method should be elucidated further or supported by pertinent references.

Dear Reviewer,

Thank you for your feedback regarding the mention of "YEKTA TAJHIZ" in section 3.5 of our manuscript. We have addressed your concerns by adding the city and country, as well as specifying the lot number of the manufacturer. Additionally, we have elaborated on the working method and provided pertinent references to support our approach.

5. Part 3.9 necessitates a more detailed account of the working method. Additionally, it is advisable to specify the type of kit used in this section.

Thank you, the method for measuring the activity of caspases has been rewritten. And the kit used was also mentioned.

Results

6. Regarding the results section, a revision is required for the initial sentence in section 4.2, line 171 to ensure accuracy and clarity.

Thanks, Necessary corrections were made.

7. When denoting significant increases or decreases in the results section, it is important to include the specific p-values in the text, as opposed to only representing them with asterisks in the captions of the graphs.

Thank you. Based on the honorable reviewer's feedback, p-values were included in the text. Given the extensive results in the article, p-values were added specifically for the combined drug groups (curcumin and etoposide) to maintain clarity and avoid overcrowding the text.

8. Enhancements in the quality and resolution are needed for the graphs and shapes, particularly in Figure 1, to meet the requisite standards of clarity and legibility.

Thanks, this problem is due to the many results of the article. The resolution of the images is high, and after the article is in PDF format, you can view the images with a high zoom without losing quality. Also, if you zoom in the Word file, the quality is high and you can see the details more accurately.

9. In Figure 3, detailing the magnification levels in parts A and B will facilitate improved interpretation and comparison of the results.

Thanks, this problem is due to the many results of the article. The resolution of the images is high, and after the article is in PDF format, you can view the images with a high zoom without losing quality. Also, if you zoom in the Word file, the quality is high and you can see the details more accurately.

Discussion

10. It is recommended to expand the discussion section by integrating relevant literature for comparisons, outlining the implications of the study findings, addressing potential limitations, and proposing avenues for future research.

Dear Reviewer,

Thank you for your valuable feedback on the discussion section of our manuscript. We have taken your suggestion to heart and have expanded the discussion to include relevant literature for comparisons, outline the implications of the study findings, address potential limitations, and propose avenues for future research.

Conclusion

11. The conclusion should be succinct yet impactful, encapsulating the significant outcomes of the study and emphasizing the relevance and applications of the research findings to the scientific community.

Dear Reviewer,

Thank you for your constructive feedback on the completion of our manuscript. We have carefully considered your proposal and revised the conclusion to make it more concise and effective. The revised conclusion now summarizes the main findings of the study and emphasizes the relevance and application of the research findings to the scientific community. We hope that these changes meet your expectations and improve the overall quality of the manuscript. Thank you again for your valuable contribution.

References

12. In the discussion section, certain references, should be substituted with more pertinent and current sources (particularly within the last 5 years).

Thank you. According to the opinion of the honorable reviewer, references in the discussion and introduction sections were limited to articles published within the last five years.

Reviewer 2:

The study's lack of novelty makes it unfit for publishing in its current state. The research specifically focuses on subjects that have been thoroughly discussed in current literature.

Response to Reviewer 2

Thank you for your insightful comments regarding the novelty of our research. We recognize the importance of distinguishing our study from existing literature and appreciate the opportunity to further elucidate the unique contributions and implications of our findings.

While it is true that the combination of chemotherapeutic agents with natural compounds has been explored, our study specifically investigates the synergistic interaction between Etoposide (ETO) and Curcumin (Cur) in breast cancer (BC) cell lines MCF-7 and MDA-MB-231. The novelty of our research lies not only in the specific combination of these compounds but also in the detailed mechanistic insights provided, which have not been comprehensively demonstrated in prior studies with these particular cell lines. Our research contributes unique findings regarding differential cellular responses and the potentiation of ETO's efficacy by Cur, underpinned by molecular markers such as p53, Bax, caspases, and Bcl-2, offering new data on their expression and

interaction. Our study highlights the varied responses of different BC cell lines to the Cur+ETO combination, with a notable reduction in IC50 values for ETO in MCF-7 cells and distinct apoptotic rates in both cell lines. This differential response underscores the importance of cellular context in therapeutic outcomes, a crucial aspect that could influence future clinical applications and drug development strategies. The enhancement in apoptotic markers and the inhibition of cell migration shown in our study provide a deeper understanding of the cellular mechanisms at play, which could lead to more targeted and effective therapeutic strategies. The detailed investigation into the molecular pathways affected by the Cur+ETO combination, particularly the roles of p53, Bax, caspases, and Bcl-2, provides significant mechanistic insights that are not thoroughly explored in existing literature. Our findings suggest a robust synergistic interaction that enhances apoptosis and inhibits migration, adding valuable knowledge to the field of cancer therapeutics. Our research contributes to the growing body of knowledge that supports the use of combination therapies in cancer treatment. By demonstrating that the combination of Cur and ETO can lead to enhanced therapeutic efficacy and potentially lower doses of chemotherapeutic agents, our study has significant implications for reducing side effects and improving patient outcomes in cancer therapy. We acknowledge the need for further research to fully elucidate the potential of this combination therapy. Our findings lay the groundwork for subsequent studies and clinical trials that could lead to the development of more effective treatment regimens with fewer side effects for patients with breast cancer. The prospect of translating these findings into clinical practice is promising and warrants further exploration. In conclusion, while the thematic foundation of our study aligns with ongoing research trends, the specific contributions, particularly in terms of mechanistic insights and differential cellular responses in BC cell lines, provide new and valuable information to the scientific community. We believe that these findings justify the publication of our study and its potential to impact future research and therapeutic strategies in oncology.

2 Revision 1

2 comments · 23 Sep 2024 – 24 Sep 2024

Referee 1 Reviewer

ACCEPT 23 Sep 2024

Thank you for your attention to all the points. All the comments have been answered correctly.

Associate Editor

ACCEPT 24 Sep 2024

The authors improved their manuscript according to the reviewers' comments and provided thoughtful answers to the questions. In my opinion, this manuscript is now satisfactory, and I recommend it for publication in IJ Pharmaceutical Research.