Iranian Journal of Pharmaceutical Research
Volume 23 · Issue 1 · e151714

OPEN PEER REVIEW

Dendrosomal Curcumin Showed Cytotoxic Effects on Breast Cancer Cell Line by Inducing Mitochondrial Apoptosis Pathway and Cell Division Arrest

Houriye Abbasi, Fatemeh Hosseinkhani, Bahareh Imani Fouladi, Siroos Tarighi, Majid Sadeghizadeh, Maryam Montazeri

SUBMITTED	REVISED	ACCEPTED	DOI
27 Jul 2024	24 Aug 2024	1 Sep 2024	10.5812/ijpr-151714

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.

More about open peer review: brieflands.com/knowledgebase/opr

Contents

1 **Original submission** Referee 1, Referee 2, Associate Editor 2
2 **Revision 1** Associate Editor 4
Authors' reply to reviewers 5
Reviewer and editor comments 9

1 Original submission

3 comments · 1 Aug 2024 – 7 Aug 2024

Referee 1 Reviewer

MINOR REVISION 1 Aug 2024

Overall, the paper is well-structured and presents its findings in a clear and logical manner, which is essential for scientific communication. The authors have included a comprehensive set of data and research to support their claims about the anti-tumor effects of dendrosomal curcumin (DNC) on breast cancer cells.

It is, however, important to note that there are a few questions and areas to be explored further:

1. The safety profile and potential toxicity of DNC, especially at the concentrations used in the study, are not addressed. This information would be crucial for considering DNC as a therapeutic agent.

2. While the authors discuss the modulation of gene and protein expression, a more detailed exploration of the exact mechanisms by which DNC induces apoptosis and cell cycle arrest could be beneficial.

Besides that, there are several grammatical errors that should be corrected prior to resubmitting the document.

Referee 2 Reviewer

MAJOR REVISION 6 Aug 2024

Manuscript ID: 151714: Dendrosomal curcumin showed anti-tumor effects on breast cancer by inducing mitochondrial apoptosis pathway.
The manuscript contains a lot of technical and substantive errors. It requires thorough improvement.

There are several major issues that need to be clarified:

1. The authors need to ensure that appropriate words that align with the context are used. In addition, the general language of the manuscript should be reconsidered.

2. The authors only assessed the effect of dendrosomal curcumin on a cell line (anti-tumor effects on breast cancer). Please revise the title of the manuscript to be in accordance with the research objective and results.

3. The anticancer effect of dendrosomal curcumin has been investigated in various in vivo and in vitro studies, including studies on the cell line used in this study. Please describe the novelty of the present research in one paragraph in the introduction section.

4. The rationale for the concentration employed must be given.

5. Please explain how the authors chose the treatment times. For example, for cell viability, lymphocytes were treated for 48 h, but the remaining experiments were performed on cells incubated for 24, 48 and 72 h. Why?

6. In the Materials and Methods section, please revise and complete the method for the cell viability assay.

7. Please provide a detailed method for Uptake kinetics in the material and methods section.

8. What were the housekeeping gene and protein for PCR and Western blot? You have to normalize your results based on a housekeeping gene or protein.

9. The results of the cell viability assay were missing. Please add the cell viability results to the results section.

10. Please provide the image of the Western blot assay that also shows the bands of the housekeeping gene.

11. The authors should work on the discussion and ensure that the focus is on discussing their results in light of what is known.

Associate Editor

MAJOR REVISION 7 Aug 2024

Review for IJPR-151714
Reviewer 1:
Manuscript ID: 151714: Dendrosomal curcumin showed anti-tumor effects on breast cancer by inducing mitochondrial apoptosis pathway.
The manuscript contains a lot of technical and substantive errors. It requires thorough improvement.
There are several major issues that need to be clarified:
1. The authors need to ensure that appropriate words that align with the context are used. In addition, the general language of the manuscript should be reconsidered.
2. The authors only assessed the effect of dendrosomal curcumin on a cell line (anti-tumor effects on breast cancer). Please revise the title of the manuscript to be in accordance with the research objective and results.
3. The anticancer effect of dendrosomal curcumin has been investigated in various in vivo and in vitro studies, including studies on the cell line used in this study. Please describe the novelty of the present research in one paragraph in the introduction section.
4. The rationale for the concentration employed must be given.
5. Please explain how the authors chose the treatment times. For example, for cell viability, lymphocytes were treated for 48 h, but the remaining experiments were performed on cells incubated for 24, 48, and 72 h. Why?
6. In the Materials and Methods section, please revise and complete the method for the cell viability assay.
7. Please provide a detailed method for uptake kinetics in the Materials and Methods section.
8. What were the housekeeping gene and protein for PCR and Western blot? You have to normalize your results based on a housekeeping gene or protein.
9. The result of the cell viability assay was missing. Please add the result of the cell viability assay to the results section.
10. Please provide the image of the Western blot assay that also shows the bands of the housekeeping gene.
11. The authors should work on the discussion and ensure that the focus is to discuss their results in the light of what is known.
Reviewer 2:
Overall, the paper is well-structured and presents its findings in a clear and logical manner, which is essential for scientific communication. The authors have included a comprehensive set of data and research to support their claims about the anti-tumor effects of dendrosomal curcumin (DNC) on breast cancer cells.
It is, however, important to note that there are a few questions and areas to be explored further:
1. The safety profile and potential toxicity of DNC, especially at the concentrations used in the study, are not addressed. This information would be crucial for considering DNC as a therapeutic agent.
2. While the authors discuss the modulation of gene and protein expression, a more detailed exploration of the exact mechanisms by which DNC induces apoptosis and cell cycle arrest could be beneficial.
Besides that, there are several grammatical errors that should be corrected prior to resubmitting the document.

2

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 Chief Editor

Thank you very much for your e-mail having the referee's comments about our article entitled "Dendrosomal curcumin showed anti-tumor effects on breast cancer by inducing mitochondrial apoptosis pathway". In response to the comments and suggestions in your letter, I would like to thank you and the referees for your valuable point of views and suggestions. Our changes in the text in response to respected referee are highlighted. Q is the question or suggestion raised by each of the respected referee and *Answer* is our explanation in response to the respected referee's suggestion.

Response to respected reviewer #1:

Q1. The authors need to ensure that appropriate words that align with context are used. In addition, the general language of the manuscript should be re-considered

Answer: The manuscript was edited grammatically and typologically.

Q2. The authors only assessed the effect of dendrosomal curcumin on a cell line (anti-tumor effects on breast cancer). Please revise the title of the manuscript to be in accordance with the research objective and results.

Answer: The title was revised and highlighted by red color.

Q3. The anticancer effect of Dendrosomal curcumin was investigated in various invivo and invitro studies, and also on cell line which used in this study. Please provide the novelty of present research in one paragraph in introduction section.

Answer: The novelty of current study is the investigation of action mechanism of this nanoformulation in breast cancer by focusing on the apoptotic pathway and mitotic cell division. We added one paragraph in Introduction section of MS and highlighted by red color.

Q4. The rational for employed concentration must be given.

Answer: The rational for used dosage is the research done by Moradi et al (2015) on the effects of DNC on MCF7 cell line. This study was cited in material and methods section and highlighted by red color.

Q5- Please explain how the authors choose treatment times. For example: for cell viability, lymphocytes were treated for 48h, but the remaining experiments were performed on cells incubated for 24, 48 and 72h. Why?

Answer: All the time trend was during 72 h hours and as seen in Figure 1 b, The viability of cells was measured during 72 h. In method section, we corrected the time (3days) and highlighted by red color.

Q6- In Materials and Methods section: please revise and complete the method for cell viability assay.

Answer: This section was corrected and highlighted by red color.

Q7- Please provide detailed method for Uptake kinetics in material and methods section

Answer: The detailed method of curcumin uptake by cells was provided in section 2.1. of methods and highlighted by red color. Also, the reference used for this cited in MS.

Q8- What was the housekeeping gene and protein for PCR and Western blot?? you have to normalize your results based on a housekeeping gene or protein.

Answer: For gene expression section, we used GAPDH gene as housekeeping gene and the primer sequence were provided in section 2.5 of MS. For western blot, we used β-actin as control protein.

Q9- The result of cell viability assay was missing. Please add the result of cell viability in results section.

Answer: The cell viability results were provided in Figure 2 along with the apoptosis and necrosis results.

Q10- Please provide the image of western blot assay which also show the bands of housekeeping gene

Answer: The bands related for B-actin for western blot assay were added in Figure 10. All the studied gene expressions were normalized by GAPDH gene as the housekeeping gene.

Q11-. The authors should work on the discussion and ensure that the focus is to discuss their results in the light of what is known.

Answer: In discussion section, we discussed all findings with the related studies.

Response to respected reviewer #2

Q: Overall, the paper is well-structured and presents its findings in a clear and logical manner, which is essential for scientific communication. The authors have included a comprehensive set of data and research to support their claims about the anti-tumor effects of dendrosomal curcumin (DNC) on breast cancer cells.

It is however important to note that there are a few questions and areas to be explored further:

Answer: We would like to thank the respected referee for his/her encouraging point of view.

Q1- The safety profile and potential toxicity of DNC, especially at the concentrations used in the study, are not addressed. This information would be crucial for considering DNC as a therapeutic agent.

Answer: The dosage of DNC used in current study was selected based on the previous research done by Moradi et al (2015) and we cited the article. However, this dose seems to be safe, as the previous research showed that 4-8 g/d of curcumin is safe for human (Zeng et al, 2022; PMC9353077). 20 µM curcumin is equal to 7.288 g/L.

Q2- While the authors discuss the modulation of gene and protein expression, a more detailed exploration of the exact mechanisms by which DNC induces apoptosis and cell

cycle arrest could be beneficial. Besides that, there are several grammatical errors that should be corrected prior to resubmitting the document.

Answer: The more detailed discussion were added in MS and highlighted by red color in Discussion section of MS. The MS was corrected grammatically.

Best regards,

Dr. M M

2 Revision 1

1 comment · 1 Sep 2024

Associate Editor

ACCEPT 1 Sep 2024

The authors improved their manuscript according to reviewers' comments and addressed the remaining concerns. In my opinion, this manuscript is now suitable for publication in IJ Pharmaceutical Research.