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OPEN PEER REVIEW

Ganoderic Acid A Extract Induces Apoptosis and Upregulates Autophagy-Related Genes in NALM-6 Cell Line: A Potential Therapeutic Agent for Acute Lymphoblastic Leukemia

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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.

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

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1 Original submission

3 comments · 23 Dec 2023 – 28 Jan 2024

Referee 1 Reviewer

MAJOR REVISION 23 Dec 2023

Dear AE,
There are some comments that need to be checked and addressed by the authors.

1. The sample size is not clear in the abstract.
2. Define the groups in the abstract.
3. Report exact P-values in the abstract.
4. What do you mean by “Fisher's post hoc test”?
5. Please report mean ± SD and exact P-values in a table.

Kind Regards

Associate Editor

MAJOR REVISION 28 Jan 2024

Dear AE,
There are some comments that need to be checked and addressed by the authors:

In general, the authors evaluated the effect of Ganoderic Acid A Extract on apoptosis and autophagy processes in the NALM-6 leukemia cell line.

These are my comments:

From the editorial point of view, the article's flow and progression were reasonable; however, I encountered several typographical errors and grammar and writing issues throughout the text. Please double-check the whole text for revision.

- The title reflects the manuscript content.

- The abstract is adequate, describing the message of the experiments.
Correct this weird sentence: “The results of the MTT test indicated that the half maximum inhibitory concentration (IC50) of leukemic cells was 140 µg/mL after 48 hours of treatment with GAA.” Do you mean the IC50 of leukemic cells or GAA?

- Introduction: It was adequately described.

- M&M: It was described in a reproducible way.
Sections 2.3. and 2.4. should be merged.
Section 2.6. should be mentioned before 2.5. to keep the connection between RNA extraction, cDNA synthesis, and qPCR.

- Results are described in a proper way.
In the sentence “Figure 2C shows NALM-6 cells treated with L-asparaginase as the positive control, which had a significantly higher apoptosis rate of 33.4% compared to the negative control and DMSO treated cells ($P < 0.0.0112$; Fig. 2)”, what is this P value? Is it a typographical error?

In figure 5, the LC3 that is recruited to the autophagosomal membrane should be LC3II.

The last sentence of the results section, “Our study sheds new light on the potential mechanisms underlying GAA's therapeutic effects in leukemia.”, should be removed from the results and moved to the conclusion.

Discussion: It is justified by the data and includes comparisons with similar literature.
The authors have included the sentence: “Activating autophagy can also promote leukemogenesis and drug resistance, making it a potential therapeutic target for leukemia treatment (Fig. 4) [17, 18].” Later, they showed that GAA treatment induced autophagy with potential therapeutic capacity. You must justify this…
Please discuss further how increasing autophagy can be a double-edged sword depending on the timing of treatment application…

- References are up to date and consistent.

Overall comments:
The scientific content of the manuscript was interesting and worth reporting for the health care system and drug development after double-checking the whole text for writing errors and applying the mentioned corrections with critical consideration.
It has merit for publication after minor modification.

Kind Regards

Editor-in-Chief

MAJOR REVISION 28 Jan 2024

Dear Author,
We have reviewed your manuscript carefully. This is to inform you that this manuscript needs major revision.
Kind Regards
EIC

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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.

We would like to thank the reviewers for careful and thorough reading of this manuscript and for the thoughtful comments and constructive suggestions, which help to improve the quality of this manuscript.

Reviewers' Comments:

Reviewer 1:
1. The sample size is not clear in the abstract.

This study was conducted in vitro and on a cell line. Various groups were determined in the abstract.

2. Define the groups in the abstract.

Suggested changes were included in the abstract.

3. Report exact P-values in the abstract.

Exact P-values are included in the abstract.

4. What do you mean by “Fisher's post hoc test”?

Fisher's post hoc test was changed to Fisher's exact test.

5. Please report mean± SD and exact P-values in a table.

I need to explain that in assessing the expression levels of genes, the expression of each of them was measured three times, and The median fold change of expression and reported as the median fold change of expression. If we refer to similar articles that have measured gene .expression, the average number of changes and the p-value are reported

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Associate Editor's Comments:

Associate Editor 1:
In general, the authors evaluated the effect of Ganoderic Acid A Extract on apoptosis and autophagy processes in NALM-6 leukemia cell line.

These are my comments:

From the editorial point of view, the article flow and path was reasonable, however, I encountered several typo mistakes and grammar and writing issues throughout the text. Please double-check the whole text for revision.

- The title reflects the manuscript content.

- Abstract is adequate, describing the message of the experiments.
Correct this weird sentence: The results of the MTT test indicated that the half maximum inhibitory concentration (IC50) of leukemic cells was 140 µg/mL after 48 hours of treatment with GAA. You mean IC50 of leukemic cells?? Or GAA?

IC50 of GAA against leukemic cells was calculated. The changes were applied in the text.

- Introduction: was adequately described.

- M&M: was described in reproducible way.
Titer 2.3. and 2.4. should be merged.
Titer 2.6. should be mentioned before 2.5. to keep the connection between RNA extraction, cDNA synthesis and qPCR.

Suggested corrections were made.

- Results are described in proper way.
The sentence: “Figure 2C shows NALM-6 cells treated with L-asparaginase as the positive control, which had a significantly higher apoptosis rate of 33.4% compared to the negative control and DMSO treated cells (P < 0.0.0112; Fig. 2)”. What is this P value? Probably typo mistake???

Thank you for pointing out the error in the P-value. The correct value is P < 0.001.

In figure 5, the LC3, which is recruited to the autophagosomal membrane should be LC3II.

Thank you! We found your comments extremely helpful and have revised accordingly.

The last sentence of result section: “Our study sheds new light on the potential mechanisms underlying GAA's therapeutic effects in leukemia.” Should be removed from results and moved to the conclusion.

Suggested corrections were made.

Discussion: It is on the data justified, compared with other similar literatures.

Authors have mentioned the sentence: “Activating autophagy can also promote leukemogenesis and drug resistance, making it a potential therapeutic target for leukemia treatment (Fig. 4) [17, 18].” And later they showed that GAA treatment induced autophagy with potential capacity for therapy. You must justify this…
Please discuss more on the issue that increasing autophagy can be a double-edge sword depend on the time of treatment application…

In the article, the opinion of the respected reviewer was considered and referenced.

- References are up to date and consistent.

Overall comments:
The scientific content of the manuscript was interesting and worth reporting for the health care system and drug development after double-checking the whole text for the writing errors and applying mentioned corrections with critical consideration.
It has merit for publication after minor modification.

2 Revision 1

3 comments · 24 Aug 2024 – 27 Aug 2024

Referee 1 Reviewer

MINOR REVISION 24 Aug 2024

Dear AE,
There are some comments that need to be checked and addressed by the authors:

The sample size is not clear in the abstract.
I didn't find any table in the manuscript.
Kind Regards

Associate Editor

MINOR REVISION 27 Aug 2024

Dear EIC,
Based on the reviewers' comments, this manuscript needs minor revision.

The issue with figure 5 is still there. The LC3 molecules that are shown on
the autophagosome membrane should be changed to LC3II.
The image shows LC3-I, which is the cytosolic form of LC3, and this is incorrect. As
you may know, the membranous form of this molecule is LC3-II.
Kind Regards
AE

Respectfully, since the authors probably did not understand what I meant, I will also explain in Persian: the molecules
located on the autophagosome must be LC3-II. This must be corrected in the figure.

Editor-in-Chief

MINOR REVISION 27 Aug 2024

Dear Author,
We have reviewed your manuscript carefully. This manuscript needs minor revision due to the reviewers' comments.
Kind Regards
EIC

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Revision 2

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.

We would like to thank the reviewers for careful and thorough reading of this manuscript and for the thoughtful comments and constructive suggestions, which help to improve the quality of this manuscript.

Reviewers' Comments:

Reviewer 1:
1. The sample size is not clear in the abstract.

> This study was conducted in vitro and on a cell line. Various groups were determined in the abstract.

2. In figure 5, the LC3, which is recruited to the autophagosomal membrane should be LC3II.

> Thank you! We found your comments extremely helpful and have revised accordingly.

3 Revision 2

3 comments · 18 Sep 2024 – 19 Oct 2024

Referee 1 Reviewer

MINOR REVISION 18 Sep 2024

Dear AE,
There are some comments that need to be checked and addressed by the authors:

1. The sample size is not clear in the abstract.
2. Report the mean ± SD in Table 2.

Kind Regards

Associate Editor

ACCEPT 19 Oct 2024

Dear EIC,
As suggested by the reviewers, this manuscript is ready to be considered for publication.
Kind Regards
AE

Editor-in-Chief

MINOR REVISION 19 Oct 2024

Dear Author,
We have reviewed your manuscript carefully. This manuscript needs minor revision due to the reviewers' comments.
Kind Regards
EIC

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Revision 3

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.

We would like to thank the reviewers for careful and thorough reading of this manuscript and for the thoughtful comments and constructive suggestions, which help to improve the quality of this manuscript.

Reviewers' Comments:

Reviewer 1:
1. The sample size is not clear in the abstract.

This study was conducted in vitro and on a cell line. 20×10^3 NALM-6 cells were cultured in triplicate across various conditions.

4 Revision 3

2 comments · 22 Feb 2025 – 26 Feb 2025

Associate Editor

ACCEPT 22 Feb 2025

Dear EIC,
As suggested by the reviewers, this manuscript is ready to be considered for publication.
Kind Regards
AE

Editor-in-Chief

ACCEPT 26 Feb 2025

Dear Authors,
This is to inform you that your manuscript has been accepted for publication.
Kind Regards
EIC