Comprehensive Health and Biomedical Studies
Volume 3 · Issue 4 · e163408

OPEN PEER REVIEW

Resveratrol Enhances Chemosensitivity of AGS Gastric Cancer Cells to Doxorubicin: Focus on Drug Resistance Mechanisms

Hakimeah Ghorbani, Kiumarth Amini, Behdokht Jamali, Siroos Tarighi, Morteza Nabiee, Afsaneh Esfandi, Zahra Damrodi

SUBMITTED	REVISED	ACCEPTED	DOI
31 May 2025	19 Jul 2025	13 Oct 2025	10.5812/chbs-163408

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, Associate Editor, Editor-in-Chief 2

2 **Revision 1** Referee 2, Referee 1, Associate Editor, Editor-in-Chief 5

Authors' reply to reviewers 6

Reviewer and editor comments 9

1 Original submission

3 comments · 9 Jun 2025 – 8 Jul 2025

Referee 1 Reviewer

MAJOR REVISION 9 Jun 2025

Reviewer Comments to the Authors:
Dear Authors,
Thank you for submitting your manuscript entitled "The Impacts of Resveratrol on Chemosensitivity of AGS Cell Line Treated with Doxorubicin – Focus on Action Mechanism" to the journal. The study addresses a relevant and timely topic in the field of cancer therapeutics and presents interesting findings on the ability of resveratrol (RSV) to enhance chemosensitivity in gastric cancer cells.
Overall, the manuscript has potential for publication; however, some revisions are required to improve the clarity, scientific rigor, and presentation of your findings. Below are my detailed comments:

Major Points for Revision:
1. Title:
o Please correct the grammar and make it more concise. Suggested revision:
"Resveratrol Enhances Chemosensitivity of AGS Gastric Cancer Cells to Doxorubicin: Focus on Drug Resistance Mechanisms"
2. Abstract:
o It lacks precise data (e.g., IC50 values, fold changes in gene expression). Please include key quantitative findings.
o Clarify that the gene expression results are from RT-PCR, not qPCR, unless SYBR Green/fluorescence was used.
3. Introduction:
o The introduction is comprehensive but would benefit from clearer transitions between ideas and better organization.
o Please provide more specific background on why AGS cells were selected and how this model represents gastric cancer.
4. Methods:
o Indicate whether RSV and Dox treatments were done in serum-free or complete medium.
o Clarify if RT-PCR results were semi-quantitative or real-time (qRT-PCR), and how gene expression was normalized.
5. Results:
o Include the exact fold changes in gene expression and not just qualitative statements like "increased" or "decreased".
6. Discussion:
o Expand the discussion on how RSV mechanistically suppresses MDR1/MRP1/MRP2, perhaps referencing SIRT1 or NF-κB if relevant.
o Include a short paragraph on the limitations of the study (e.g., in vitro-only model, need for in vivo validation).
o Suggest potential for clinical translation or combination therapy protocols.
7. Conclusion:
o Rephrase to be more concise and scientific. Avoid using general terms like "it seems"—be assertive with evidence-based conclusions.

Associate Editor

MAJOR REVISION 8 Jul 2025

Reviewer 1 Comments to the Authors:
Dear Authors,
Thank you for submitting your manuscript entitled "The Impacts of Resveratrol on Chemosensitivity of AGS Cell Line Treated with Doxorubicin – Focus on Action Mechanism" to the journal. The study addresses a relevant and timely topic in the field of cancer therapeutics and presents interesting findings on the ability of resveratrol (RSV) to enhance chemosensitivity in gastric cancer cells.
Overall, the manuscript has potential for publication; however, some revisions are required to improve the clarity, scientific rigor, and presentation of your findings. Below are my detailed comments:
Major Points for Revision:
1. Title:
o Please correct the grammar and make it more concise. Suggested revision:
"Resveratrol Enhances Chemosensitivity of AGS Gastric Cancer Cells to Doxorubicin: Focus on Drug Resistance Mechanisms"
2. Abstract:
o It lacks precise data (e.g., IC50 values, fold-changes in gene expression). Please include key quantitative findings.
o Clarify that the gene expression results are from RT-PCR, not qPCR, unless SYBR Green/fluorescence was used.
3. Introduction:
o The introduction is comprehensive but would benefit from clearer transitions between ideas and better organization.
o Please provide more specific background on why AGS cells were selected and how this model represents gastric cancer.
4. Methods:
o Indicate whether RSV and Dox treatments were done in serum-free or complete medium.
o Clarify if RT-PCR results were semi-quantitative or real-time (qRT-PCR), and how gene expression was normalized.
5. Results:
o Include the exact fold changes in gene expression and not just qualitative statements like "increased" or "decreased".
6. Discussion:
o Expand the discussion on how RSV mechanistically suppresses MDR1/MRP1/MRP2, perhaps referencing SIRT1 or NF-κB if relevant.
o Include a short paragraph on the limitations of the study (e.g., in vitro-only model, need for in vivo validation).
o Suggest potential for clinical translation or combination therapy protocols.
7. Conclusion:
o Rephrase to be more concise and scientific. Avoid using general terms like "it seems"—be assertive with evidence-based conclusions.
Riviewer 2:
Thank you for submitting the manuscript on “The impacts of Resveratrol on chemosensitivity of AGS cell line treated with doxorubicin-Focus on action mechanism” for review. Please consider the following comments to improve the quality of the manuscript.

1- It would be better to specify which company supplied Doxorubicin.

2- It would be better to mention the concentrations of Doxorubicin and resveratrol used in the materials and methods section.

Editor-in-Chief MAJOR REVISION 8 Jul 2025

Major Revision

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 "The impacts of Resveratrol on chemosensitivity of AGS cell line treated with doxorubicin-Focus on action mechanism". 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. Please correct the grammar and make it more concise. Suggested revision:

"Resveratrol Enhances Chemosensitivity of AGS Gastric Cancer Cells to Doxorubicin: Focus on Drug Resistance Mechanisms"

Answer: The title of article has changed according to the suggestion and highlighted.

Q2. 2. Abstract:

o It lacks precise data (e.g., IC50 values, fold-changes in gene expression). Please include key quantitative findings.

Answer: The IC50 and Fold change quantitative results were added in results section of abstract.

o Clarify that the gene expression results are from RT-PCR, not qPCR, unless SYBR Green/fluorescence was used.

Answer: The gene expression data were measured by RT-PCR and mentioned in abstract.

3. Introduction:

o The introduction is comprehensive but would benefit from clearer transitions between ideas and better organization. Please provide more specific background on why AGS cells were selected and how this model represents gastric cancer.

Answer: the most widely used cell model in gastric cancer research is the AGS cell line, and it is of great importance in studies related to tumor growth, invasion, and metastasis, especially in evaluating drug effects and developing new therapeutic approaches for gastric cancer (23-25). For this reason, this gastric cancer cellular model was used in the present study. This was added in last paragraph of introduction section and highlighted.

4. Methods:

Indicate whether RSV and Dox treatments were done in serum-free or complete medium.

Answer: The cell treatments were done in complete medium supplemented with 5% FBS (reduced-serum medium) and antibiotics.

Clarify if RT-PCR results were semi-quantitative or real-time (qRT-PCR), and how gene expression was normalized.

Answer: The results presented are qRT-PCR and the normalization of gene expression data are described in gene expression sub-section of MS. We used GAPDH gene as the control housekeeping gene and all data of gene expression were normalized based on this gene.

5. Results:

Include the exact fold changes in gene expression and not just qualitative statements like "increased" or "decreased"..

Answer: All the gene expression data were included in the results and highlighted by red color.

6. Discussion:

Expand discussion on how RSV mechanistically suppresses MDR1/MRP1/MRP2, perhaps referencing SIRT1 or NF-κB if relevant.

Answer: We added this suggestion in discussion section and highlighted by red color.

Include a short paragraph on limitations of the study (e.g., in vitro-only model, need for in vivo validation).

Answer: We added the limitation of study in last paragraph of discussion section of MS.

Suggest potential for clinical translation or combination therapy protocols.

Answer: We added this in last sentence of discussion.

7. Conclusion:

Rephrase to be more concise and scientific. Avoid using general terms like "it seems"—be assertive with evidence-based conclusions.

Answer: The conclusion section was concise.

Response to respected reviewer #2

Q: Thank you for submitting the manuscript on "The impacts of Resveratrol on chemosensitivity of AGS cell line treated with doxorubicin-Focus on action mechanism" for review. Please consider the following comments to improve the quality of the manuscript.

1 -It is better to find out from which company Doxorubicin was obtained.

Answer: Both RVS and Dox were purchased from Sigma Corp. We added this in materials and Methods section.

2 -It is better to mention the used concentrations of Doxorubicin and resveratrol in the materials and methods section.

Answer: The concentrations of RSV and Dox were added in materials and methods section.

Best regards,

Dr. Behdokht Jamali

2 Revision 1

4 comments · 14 Sep 2025 – 13 Oct 2025

Referee 2 Reviewer

MAJOR REVISION 14 Sep 2025

Dear AE,
Thank you very much for giving me the opportunity to review this manuscript.
This experimental study investigates a highly relevant and translational research question in oncology: overcoming chemoresistance in gastric cancer. The focus on resveratrol (RSV), a natural compound with known chemopreventive properties, as a potential chemosensitizer to doxorubicin (Dox) is rational and interesting. The study employs standard in vitro methodologies (cell culture, MTT assay, RT-PCR) to assess cell viability and expression of key genes involved in apoptosis (Bcl-x) and multidrug resistance (MDR1, MRP1, MRP2). The reported findings—that RSV co-administration reduces cell viability and downregulates anti-apoptotic and drug efflux genes—are promising and align with the hypothesized mechanism. However, the manuscript, as presented in the abstract, lacks critical methodological details, appropriate statistical reporting, and sufficient mechanistic depth to robustly support its conclusions. The results are presented in a way that raises significant questions about the experimental design and data interpretation.

1. The IC50 for RSV is reported (50 µg/mL), but the IC50 for Dox alone is conspicuously absent. This is a fundamental requirement for a chemosensitization study. Furthermore, the concentration of Dox used in the combination treatment is not stated. Was it a sub-optimal dose? The full dose-response curves for both drugs alone and in combination are needed to calculate a Combination Index (CI) using models like Chou-Talalay. This would quantitatively demonstrate whether the interaction is additive, synergistic, or antagonistic. The claim of "improved chemosensitivity" is weak without this formal analysis.
2. The duration of treatment for both the MTT and RT-PCR experiments is not specified. This is a critical parameter, as effects on viability and gene expression can be highly time-dependent.
3. The abstract lists p53 as a gene that was evaluated, but no result is reported for it. This is a major omission, as p53 is a central regulator of apoptosis and cell cycle arrest and its status (wild-type or mutant) in AGS cells can dictate the response to genotoxic stress like Dox. The results for p53 must be included and discussed.
4. The gene expression data are presented as "fold change" with a mean and standard deviation (e.g., MRP1: 0.42 ± 0.07). However, what are they compared to? The control should be clearly defined (e.g., untreated cells? Dox-only treated cells?). Most importantly, no p-values are provided to indicate whether these changes are statistically significant. A fold change of 0.56 for Bcl-x is biologically interesting, but without a measure of statistical significance, its importance cannot be assessed. All quantitative results must be accompanied by appropriate statistical tests (e.g., one-way ANOVA with a post-hoc test) and p-values.
5. The abstract does not state the number of biological replicates (independent experiments) performed for the MTT or RT-PCR assays. Were the experiments performed in triplicate? This is essential for assessing the reliability of the data.
6. The study only measures mRNA expression by RT-PCR. Changes in mRNA do not necessarily translate to changes in functional protein levels, which are the ultimate mediators of drug efflux (MDR1/P-gp, MRP1/2) and apoptosis inhibition (Bcl-xL). Western blot analysis to confirm the downregulation at the protein level is strongly recommended to solidify the proposed mechanism.
7. The hypothesis that downregulation of MDR1 and MRP genes leads to increased intracellular Dox accumulation is compelling but was not directly tested. A simple Dox accumulation/retention assay using its intrinsic fluorescence would provide direct functional evidence for the proposed mechanism of chemosensitization.
8. The downregulation of Bcl-x suggests enhanced apoptosis. This should be confirmed with a direct assay for

apoptosis (e.g., Annexin V/PI staining by flow cytometry) in the combination group compared to the single-agent groups.
9. The conclusion is overstated based on the evidence presented. The study shows associations but does not prove that the gene expression changes are the cause of the increased cytotoxicity. The language should be tempered to "suggests a potential mechanism" rather than stating it as fact.
10. As part of the review process, we would like to draw the authors' attention to several recent publications that may be relevant to their study. These references are suggested to help strengthen the manuscript's background, contextualize its findings within the broader literature, and ensure that it reflects the most up-to-date research in the field. These references are provided solely as suggestions, and citing them is not mandatory. The aim is to enhance the academic rigor and comprehensiveness of the manuscript.
These articles are listed below:
A. Thymoquinone Effects on the Expression of p62 Gene in Cisplatin-Induced Testicular Damage in Mice
* Reason: This article is highly relevant as it investigates the protective effects of a natural compound (thymoquinone) against chemotherapy-induced testicular toxicity by modulating autophagy and oxidative stress pathways, which parallels the manuscript's focus on resveratrol's role in enhancing chemosensitivity and reducing resistance in cancer cells. Both studies explore natural compounds mitigating chemotherapy-related damage through molecular mechanisms.
** Link: https://brieflands.com/articles/jjcmb-151663
B. The Combined Effects of Cisplatin and Royal Jelly on Lung Cancer: Growth, Proliferation, Gene Expression, and Apoptosis
* Reason: This article is highly relevant as it investigates the synergistic effects of a natural compound (royal jelly) with cisplatin chemotherapy on cancer cells, demonstrating enhanced apoptosis and gene expression changes—directly paralleling the manuscript's focus on resveratrol's role in improving chemosensitivity and reducing resistance in gastric cancer cells. Both studies explore natural compounds as adjuvants to chemotherapy.
** Link: https://brieflands.com/articles/ijcm-161127
C. Neuroprotection by Resveratrol in Chronic Cerebral Hypoperfusion: A Study on Synaptogenesis Enhancement and Apoptosis Inhibition
* Reason: This article is highly relevant as it directly investigates resveratrol's neuroprotective effects, including its anti-apoptotic and synaptogenic mechanisms in a cerebral hypoperfusion model. This supports the manuscript's findings on resveratrol's role in enhancing chemosensitivity and modulating apoptosis-related genes (e.g., Bcl-x) in gastric cancer cells, providing complementary evidence of its broad therapeutic potential.
** Link: https://brieflands.com/articles/ijpr-162425

Overall, the study addresses an important concept but is currently preliminary. The findings are intriguing but require substantial strengthening through more complete data reporting, rigorous statistical analysis, and a more sophisticated experimental design to prove chemosensitization and mechanism. The manuscript in its current form is not sufficient for publication. However, with the recommended revisions, it could become a solid contribution to the literature.

Final Decision: Major Revision

Kind Regards | Publishing Assistant

Referee 1 Reviewer

ACCEPT 8 Oct 2025

It is accepted.

Associate Editor ACCEPT 8 Oct 2025

accepted

Editor-in-Chief ACCEPT 13 Oct 2025

I am writing to inform you of the final decision on your manuscript, which has been accepted for publication in CHBS.
We appreciate your contribution to our journal and look forward to your continued engagement with us.

Yours sincerely,
F. Ghanbari
EIC