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OPEN PEER REVIEW

Investigation of the Antioxidant and Cellular Toxicity Activities of Gold Nanoparticles Synthesized Using *Cichorium intybus* Extract on a Liver Cancer Cell Line

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About this report

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1 Original submission

2 comments · 20 Jan 2025 – 28 Jan 2025

Referee 1 Reviewer

MINOR REVISION 20 Jan 2025

The manuscript provides a comprehensive investigation of the antioxidant and cytotoxic properties of biosynthesized gold nanoparticles (AuNPs) using Cichorium intybus extract. The biosynthesized nanoparticles were characterized using several methods, including XRD, FTIR, FESEM, EDX, and UV/Vis spectroscopy. The topic is interesting; however, the manuscript requires several minor revisions. Below are my specific comments to improve the quality of the manuscript for publication in the IJPR journal:

1. The manuscript contains numerous grammatical, vocabulary and dictation errors, and its language requires polishing. Examples include but are not limited to:

a. Please revise the title to: “Investigation of the Antioxidant and Cellular Toxicity Activities of Gold Nanoparticles Synthesized Using Cichorium intybus Extract on a Liver Cancer Cell Line."

b. Replace "remedy" with "treatment," as "treatment" is more appropriate for this context.

c. Page 1: Revise “The antioxidant consequences display” to “The antioxidant results demonstrate”.

d. Pages 5–6: Replace "shacked" with "shook," which is the correct past tense of "shake."

e. Page 17: Correct “BAX gen” to “BAX gene”.

…

2. Every abbreviation, acronym, or initialism must be written in its full form the first time it appears in the manuscript.

3. Figure captions should be concise and free of unnecessary details. Any relevant interpretation or analysis should be included in the manuscript's body rather than the captions.

4. The last paragraph of the introduction should clearly state the purpose, novelty, and significance of the study. Currently, the introduction lacks a concluding paragraph summarizing these aspects.

5. Section 2.1. Plant material, biosynthesis and characterization:

a. Specify all the materials used in the study, not just chloroauric acid. Include their chemical names, grades, and sources (e.g., Sigma-Aldrich, USA).

b. For the plant materials, please clearly identify the plant species, and provide some details about how the plant materials were processed or prepared for use.

c. Please ensure consistency in mentioning the country of origin for all instruments. Either include the country of origin for all instruments or omit it entirely. Additionally, specify the brand name of the XRD instrument used in the study.

d. How are the “powdered AuNPs” prepared? Please explain the method.

6. Section 2.2. DPPH radical scavenging test:

a. Briefly explain the mechanism of the test. Also, Line 72 is unclear and should be revised.

b. In Line 75, this formula doesn’t give the percentage of inhibition. The result should be multiplied by 100.

7. Section 2.3. Cytotoxicity of AuNPs:

a. Specify the cell culture medium and conditions.

b. What is the concentration of the MTT solution?

c. What is the number of replications for each test?

8. In section “3.1. X-ray diffraction”, please add that the high intensity of the (111) peak suggests that the gold nanoparticles exhibit a preferential orientation along this plane.

9. Fig 2: The caption indicates that the red spectrum relates to AuNPs and the blue spectrum to the extract, but the figure suggests the reverse. Please correct this inconsistency.

10. Section 3.3. Field Emission Scanning Electron Microscopy (FESEM) and Spectrophotometric Analysis:
a. There is no mention of the specific method or software used to analyze the SEM images and extract particle size information (e.g., ImageJ, MATLAB, or other tools).
b. Please describe the methodology of particle size analysis. Some information is visible in the picture but is not discussed in the body of the manuscript.
c. Please explain why a normal distribution has been used to fit this data set. Check if other distribution models (like log-normal) fit the data set better.
d. Figure 3,4: If you include two images in one figure, label them as a, b, etc. In the caption, specify which part each explanation refers to, rather than giving unclear, general descriptions.
e. Fig 4: Although the explanation should be written in the body of the manuscript instead of the caption, it is not clearly stated which graph the explanation refers to.
f. Please insert axis titles in the EDX graph in Fig 4.

11. In figure 5's caption, the percentage inhibition for BHA is stated as 94.4%, but the figure shows 92.43%. Please correct this discrepancy. Also, please note that such information should be written in the body of the manuscript instead of captions.

12. The conclusion mentions that the particle size of nanoparticles is 118 ± 72 nm. If this size is obtained using DLS, include this information in the methods and results sections. If it is not obtained using DLS, clarify the method used to determine this size.

Associate Editor

MAJOR REVISION 28 Jan 2025

It is recommended that the reviewers' comments be fully addressed in the revision.

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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,

We thanks a lot the reviewers and the editor for detailed study and valuable comments.
In the modified version of the article authors have made every effort to implement all respectable reviewers comments and have changed the article in order to meet reviewers comments.

Yours Sincerely,

The Authors

Reviewers' Comments:

Reviewer 1:

1.The manuscript needs English editing; there are a number of sentences that are non-comprehensive: e.g. evaluate the effects of the synthesized gold nanoparticles by Cichorium intybus extract the antioxidant and cellular toxicity activity …, using the employ of…, etc.

Response: Thank you very much for your detailed study. The manuscript is reconsidered again for English editing.

2. Add a paragraph at the end of the introduction to explain the importance and aim of the current study

Response: Done.

3. Add the time of plant harvesting in the methods

Response: Done. The plant materials were collected from Kashmar (north-east of Iran) in 10 May 2024.

4. At least one main active compound of the extract should be identified and quantified

Response: According to FTIR results, the phenols are main active compound of the extract. Due to the lack of grant funding and time constraints, it is not possible for us to determine the types of phenols present in the extract, but we will certainly consider it in future research.

5. First section of methods should be divided in to “materials and reagents” and “preparation of herbal extract and gold nanoparticle synthesis”

Response: Thank you for your suggestion. Done.

6. Indicate the concentration of HAuCl4 solution in methods

Response: 10 mL HAuCl4 (10 mg) salt was used. It is mentioned in the methods section.

7. The part of DPPH radical scavenging test should be rewritten

Response: According to respected reviewer comment, this section is rewritten.

8. The results subtitles are more suitable for the methods. They should be revised

Response: The results subtitles are modified.

9. Add subsections (a, b, C, ...) to the figures that have multiple results and describe them in caption

Response: Thank you very much for your suggestion. Subsections are added to the figures.

10. Add the method used for determining the NPs concentration, in methods

Response: The synthesis procedure and concentration of materials is improved.

11. Define statistical signs used in figures in their captions

Response: Done.

12. It would be better that the antioxidant and anticancer effect of crude extract and bare GNP also be added, similar to those publications discussed in the discussion

Response: Thanks a lot for your suggestion. We present the antioxidant and anticancer activity of biosynthesized GNPs, but the crude extract does not show specific activity.

13. The result of the cytotoxicity assay of the current study should be compared with the previously published similar works

Response: Table 1 is added to the article. In this table a comprehensive review of plant-mediated synthesized gold nanoparticles is done and the antioxidant and cytotoxicity activity of them are mentioned.

14. In general discussion could not properly discuss the results of this study and highlight its importance

Response: Dear reviewer, the more concise discussion is due to the limitation of words and references in the journal guidelines. However, we tried to provide satisfactory responses to the comments by adding a table, improving various sections of the methods and results, and comparing the results with other research works.

15. Discuss the limitations of the study and the future trends

Response: Section 5 with title of “limitations and the future trends “is added. Thank you very much again for your detailed study.

Reviewer 2:

The manuscript provides a comprehensive investigation of the antioxidant and cytotoxic properties of biosynthesized gold nanoparticles (AuNPs) using Cichorium

intybus extract. The biosynthesized nanoparticles were characterized using several methods, including XRD, FTIR, FESEM, EDX, and UV/Vis spectroscopy. The topic is interesting; however, it requires several minor revisions. Below are my specific comments to improve the quality of the manuscript for publication in the IJPR journal:

Response: Thank you very much for your detailed study.

1. The manuscript contains numerous grammatical, vocabulary and dictation errors, and its language requires polishing. Examples include but are not limited to:

a. Please revise the title to: “Investigation of the Antioxidant and Cellular Toxicity Activities of Gold Nanoparticles Synthesized Using Cichorium intybus Extract on a Liver Cancer Cell Line."

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c. Page 1: Revise “The antioxidant consequences display” to “The antioxidant results demonstrate”

d. Pages 5–6: Replace "shacked" with "shook," which is the correct past tense of "shake."

e. Page 17: Correct “BAX gen” to “BAX gene”

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Response: Thanks a lot for your attention. All of the above issues are corrected. Also, the article is reconsidered again for language edition.

2. Every abbreviation, acronym, or initialism must be written in its full form the first time it appears in the manuscript.

Response: list of all abbreviation is prepared and mentioned in the beginning of article.

3. Figure captions should be concise and free of unnecessary details. Any relevant interpretation or analysis should be included in the manuscript's body rather than the captions.

Response: Done.

4. The last paragraph of the introduction should clearly state the purpose, novelty, and significance of the study. Currently, the introduction lacks a concluding paragraph summarizing these aspects.

Response: Done.

5. Section 2.1. Plant material, biosynthesis and characterization:

a. Specify all the materials used in the study, not just chloroauric acid. Include their chemical names, grades, and sources (e.g., Sigma-Aldrich, USA).

b. For the plant materials, please clearly identify the plant species, and provide some details about how the plant materials were processed or prepared for use.

c. Please ensure consistency in mentioning the country of origin for all instruments. Either include the country of origin for all instruments or omit it entirely. Additionally, specify the brand name of the XRD instrument used in the study.

d. How are the “powdered AuNPs” prepared? Please explain the method.

Response: Thank you for your comment. The methods and materials section is full modified according to above comments and materials, plant species, extraction, and preparation of AuNps parts are complete explained.

6. Section 2.2. DPPH radical scavenging test:

a. Briefly explain the mechanism of the test. Also Line 72 is unclear and should be revised.

b. In Line 75, this formula doesn’t give you percent of inhibition. It should be multiplied by 100.

7. Section 2.3. Cytotoxicity of AuNPs:

a. Specify the cell culture medium and conditions.

b. What is the concentration of MTT solution?

c. What is the number of replications for each test?

Response: All of the requested items are done and corrected.

8. In section “3.1. X-ray diffraction”, please add that the high intensity of the (111) peak suggests that the gold nanoparticles exhibit a preferential orientation along this plane.

Response: Fig 1 is improved.

9. Fig 2: the caption indicates that the red spectrum relates to AuNPs and the blue spectrum to the extract, but the figure suggests vice versa. Please correct this inconsistency.

Response: Done. A and B label for this figure and other figs is added.

10. Section 3.3. Field Emission Scanning Electron Microscopy (FESEM) and Spectrophotometric Analysis:

a. There is no mention of the specific method or software used to analyze the SEM images and extract particle size information (e.g., ImageJ, MATLAB, or other tools).

b. Please write the methodology of particle size analysis. Some information are visible in the picture but are not discussed in the body of the manuscript.

c. Please explain why a normal distribution has been used to fit this data set. Check if other distribution models (like log-normal) fit the dataset better.

d. Figure 3,4: If you include two images in one figure, label them as a, b, etc. In the caption, specify which part each explanation refers to, rather than giving unclear, general descriptions.

e. Fig 4: Although the explanation should be written in the body of the manuscript instead of the caption, it is not clearly stated which graph the explanation refers to.

f. Please insert axes titles in the EDX graph in Fig 4.

Response: Thank you for your comments. Section 3.3. Field Emission Scanning Electron Microscopy (FESEM) and Spectrophotometric Analysis is fully modified. Figures 3 and 4 were placed separately for better understanding.

11. In figure 5's caption, the percentage inhibition for BHA is stated as 94.4%, but the figure shows 92.43%. Please correct this discrepancy. Also, please note that such information should be written in the body of the manuscript instead of captions.

Response: Thank you for your attention. It is corrected. The captions for all the figures have been concise and more information is added in the body of the manuscript.

12. The conclusion mentions that the particle size of nanoparticles is 118 ± 72 nm. If this size is obtained using DLS, include this information in the methods and results sections. If not from DLS, clarify the method used to determine this size.

Response: The particle size of NPs was done using FESEM images and the electronic microscopic study is explained completely in the revise version of article. Thank you very much again for your detailed study and your valuable comments.

2 Revision 1

1 comment · 8 Mar 2025

Associate Editor

MINOR REVISION 8 Mar 2025

The authors should address the comments fully before publication.

3

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.

Reviewer 1:

Please include the name of the liver cancer cell line in the abstract method section. Additionally, all scientific names should be italicized.

Response: It is added.

The method for preparing powdered gold nanoparticles is not yet clear. Does the term “powdered” indicate that the gold nanoparticles were dried?

Moreover, the method for calculating the concentration of gold nanoparticles has not yet been presented in the manuscript.

Response: Five grams of powdered leaves were added to 100 mL of deionized water and stirred continuously for 24 hours at room temperature to ensure thorough extraction of the bioactive compounds. The resulting solution was filtered using Whatman filter paper to remove any particulate matter. The clear filtrate was stored in an Erlenmeyer flask and kept at 3°C to preserve its bioactivity for subsequent nanoparticle synthesis. For the synthesis of gold nanoparticles, a stock solution was prepared by dissolving 10 mg of $HAuCl_4$ in 10 mL of deionized water. Subsequently, 10 mL of this stock solution was added to 90 mL of the herbal extract, resulting in a final volume of 100 mL. The mixture was shaken regularly at room temperature for 4 hours to facilitate the reduction of Au^{3+} ions and the formation of gold nanoparticles. The synthesized gold nanoparticles were then centrifuged at 10,000 rpm for 10 minutes to separate the nanoparticles from the reaction mixture. The supernatant was discarded, and the pellet containing gold nanoparticles was washed once with distilled water to remove any unreacted components and impurities. This washing step was repeated to ensure the purity of the nanoparticles. The washed nanoparticles were then dried in a vacuum oven at 40°C for 24 hours to obtain powdered gold nanoparticles. The dried nanoparticles were stored in an airtight container to prevent any contamination or oxidation until further use. The concentration of gold nanoparticles in the solution is approximately 50 ppm. The concentration of gold nanoparticles in the solution was calculated based on the initial amount of $HAuCl_4$ used and the final volume of the solution. Thank you very much for your detailed study.

3 Revision 2

1 comment · 3 Apr 2025

Associate Editor

ACCEPT 3 Apr 2025

The manuscript is now appropriate for publication in IJPR.