**Author’s response to reviews**

**Title:** Caffeine citrate enhanced cisplatin antitumor effects in osteosarcoma and fibrosarcoma in vitro and in vivo

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**Version:** 1  **Date:** 11 Jun 2019

**Author’s response to reviews:**

June 7, 2019

Dr. Alexandros Houssein
Editor
BMC Cancer

Dear Editor:

Thank you very much for your prompt arrangement and thorough review of our above-referenced manuscript entitled “Caffeine citrate enhanced cisplatin antitumor effects in osteosarcoma and fibrosarcoma in vitro and in vivo”.

According to the editor and reviewers' suggestions, the manuscript has now been revised. All changes to the manuscripts were indicated using tack changes. We have responded to all of the comments as follows:
[Comment 1 from Editor]

Please can you provide a link to the guideline that was followed with regards to the maximum tumour size in the mice.

Response to Comment 1

Thank you for pointing the important thing. The humane endpoints was set that the maximum tumor volume was 10% of normal body weight according to Institutional Animal Care and Use Committee (IACUC) guidelines. We added the information about the guideline that was followed with regards to the maximum tumour size in the mice in the Methods section (page 9, line 15–17).

[Comment 2 from Editor]

Please add a section "Additional files" (after the References/Figure legends) where you list the following information for each additional-supplementary file in the file inventory:

- File name (e.g. Additional file 1)
- Title of data
- Description of data

Response to Comment 2

We added a section “Additional files” in page 26.

We deeply appreciate the valuable comments from reviewer 1 and 2.

[Comment 1 from Reviewer 3]

The manuscript is not focused as it tries to investigate both osteosarcoma and fibrosarcoma in the same study.

Response to Comment 1

Thank you for the valuable comments. The main purpose of this time was to evaluate the enhancing effect of caffeine citrate, caffeine and citric acid on cisplatin. Therefore, we conducted the same experiment, although on two sarcoma cell lines with different properties. Cisplatin for osteosarcoma is the standard treatment. Cisplatin for fibrosarcoma is not a standard treatment,
but the cell line HT1080 is a widely distributed cell line, and the effect of cisplatin on this cell line was observed, so it was used in this experiment.

[Comment 2 from Reviewer 3]

If the authors want to use both the cancer models, it would have been beneficial to study the differential effects of combination therapy on fibrosarcoma and osteosarcoma. This could be addressed by looking at molecular mechanisms and some protein markers.

Response to Comment 2

Thank you for the valuable comments. We will consider to analyze molecular mechanisms and some protein markers in the future project. Thanks again.

[Comment 3 from Reviewer 3]

The conclusion section in the "Abstract" needs to be rephrased and rewritten as it reads like a statement on observed results.

Response to Comment 3

Thank you for your comments. We rephrased and rewritten the Abstract section (page 2–3).

[Comment 4 from Reviewer 3]

The manuscript has included statistical analysis. However, quantitative information (fold change or percent change) will be required for all the figures.

Response to Comment 4

Thank you for your comments. We added quantitative information (percent change) in Figure 3 and 4. And we also added the following sentences in Figure Captions section, page 24, line 20 (Figure 3) and page 25, line 1–2 (Figure 4).

Figure 3: The number in the Overlay panels is the rate of EdU-positive cells.

Figure 4: The number in the Overlay panels shows the intensity of luminance per cell when the non-drug is based on 100%.
[Comment 5 from Reviewer 3]

The "Results" should have titles and presentations explaining the observed effects. It has been titled like "Methods" section. For example, instead of presenting as "cell proliferation assay" it should be presented as "Treatment effects on cell proliferation"

Response to Comment 5

Thank you for your comments. We changed subtitles in Results section as below.

3.1 Effect of cisplatin and combinations on cell survival and combination index. (page 10, line 1)

3.2 Effect of cisplatin and combinations on cell proliferation. (page 10, line 12)

3.3 Effect of cisplatin and combinations on mitochondrial membrane potential. (page 11, line 1)

3.4 Effect of cisplatin and combinations on cell cycle profile. (page 11, line 13)

3.5 Effect of cisplatin and combinations on caspase 3/7 activity. (page 12, line 2)

3.6 Effect of cisplatin and combinations on mouse tumors. (page 12, line 15)

[Comment 6 from Reviewer 3]

There are many grammatical and typographical errors throughout the manuscript (some examples are given below) which need to be visited and edited by professional proof-reading. Some examples from the manuscript text:

a. Methods section: Page 13, line 19: change "activatity" to "activity"

b. Page 10, line 5: "Inhivited" to "inhibited"

c. Page 14, line 15: "upregurated" to "upregulated"

Response to Comment 6

Thank you for pointing those out. We corrected all errors as below. In addition, in Authors’ Contributions section, co-authors are changed to be appropriate. (Removed extra co-authors. Page 19, line 4–16)

Background section: Page 4, line 19: change "exisiting" to "existing"

Background section: Page 4, line 21: change "chemotherapt" to "chemotherapy"

Methods section: Page 8, line 4: change "activatity" to "activity"
Methods section: Page 9, line 1: change " mg/kg), citrate (100 mg/kg), and caffeine citrate (200 mg/kg). The regimen 2 (R2) " to " mg/kg), citrate (100 mg/kg), and caffeine citrate (200 mg/kg). The regimen 2 (R2) " (We erased the extra spaces.)

Methods section: Page 9, line 4: change " caffeine (50 mg/kg), citrate (50 mg/kg), and caffeine citrate (100 mg/kg)." to " caffeine (50 mg/kg), citrate (50 mg/kg), and caffeine citrate (100 mg/kg)." (We deleted the extra spaces.)

Methods section: Page 9, line 5: change "administarted" to "administrated"

Methods section: Page 9, line 10: change "euthanize" to "euthanized"

Results section: Page 10, line 4: change "inivited" to "inhibited"

Methods section: Page 11, line 25: change " combine with caffeine or citrate. (Figure 5)." to " combine with caffeine or citrate (Figure 5)." (We deleted the extra period.)

Discussion section: Page 13, line 16: change "unregurated" to "unregulated"

Figure Captions section: Page 25, line 18: change "inplanted" to "implanted"

We thank you again for your valuable comments, which have helped improve this paper. We are now confident that the revised version will satisfy your concerns, and look forward to hearing from you soon.

With best wishes and highest regards, Sincerely yours,

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