Format for ANSWERING REVIEWERS

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 9904-edited).

Title: Differential Control of Growth, Apoptotic Activity and Gene Expression in Human Colon Cancer Cells by Extracts Derived from Medicinal Herbs, Rhazya stricta and Zingiber officinale and Their Combination

Author: Ayman I. Elkady; Rania Abd El Hamid Hussein; Osama A. Abu-Zinadah

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 9904

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer I Comment:

1- Normal colon cell control is a necessary to verify the toxicity of these two compounds. It is critical for the normal cell control to be included in this manuscript.

We used other cancer and normal cell lines in a currently accepted our article "*Effects of Crude Extracts from Medicinal Herbs, Rhazya stricta and Zingiber officinale on Growth and Proliferation of Human Brain Cancer Cell Line in Vitro by Ayman I. Elkady; Rania Abd El Hamid Hussein; Osama A. Abu-Zinadah, BioMed Research International, Volume 2014 (2014) (in Press)*".

2- The concentrations of single and combined treatment are all different and it is hard to compare the effect of different concentration, it would be better if there would be one concentration overlapped. For example, in Fig 1A, it would be better if the author could include 50 μg/ml CAERS + 50 μg/ml CFEZO in the combined treatment instead of stop at 25+25 ug/ml treatment.

Actually, 25 μg/ml CAERS + 25 μg/ml CFEZO were the minimal doses for combined treatment capable of inducing apoptosis in HCT116 without emergence of necrotic cells. We did not like to continue with the combination of higher concentration, such as 50 μg/mL CAERS and 50 μg/mL CFEZO, since these higher doses showed too much cytotoxicity (necrotic cells) as we found using Giemsa staining (data not shown). Consistent with our results, it has been confirmed that increasing dose (or treatment time) of therapeutic agents may lead to secondary necrosis. Therefore, we had to select 12.5 μg/mL CAERS and 12.5μg/mL CFEZO for combined treatments in all experiments.

3- In Fig 1, there is no error bar for the first figure.

Error bar was included in the revised version.

4- For Fig 2, the authors should label the pictures more carefully in the figure, it is hard to figure out what each row means. There are (A) and (B) in the figure legend but no A,B in the figure. What is the fourth

row, why there are only four pics instead of seven? Also, the first picture of row 1 and row 2 look the same.

The pictures in Figure 2 were correctly labeled in the revised version. The fourth row is Toluidine blue-stained semithin sections. There are only 4 pictures, since these 4 pictures represent lower doses of the treatments (75 µg/mL CAERS, 75 µg/mL CFEZO and 12.5 µg/mL CAERS + 12.5 µg/mL CFEZO). When we used higher doses (e.g., 100 µg/mL CAERS, 100 µg/mL CFEZO and 20 µg/mL CAERS + 20 µg/mL CFEZO), cells could not processed for preparation of semithin sections or scanning electron microscope images.

5- For Fig 3A, why first and second row are different as they both stained with Hoechst?

The different images were replaced with the right images in the revised version. The right doses of the CAERS and CFEZO in the most right images in Fig 3A and Fig 3B were indicated.

6- The introduction part is too long with unnecessary information, it would be better if the authors could improve it.

The introduction part has been revised in the revised version.

Reviewer II Comment:

1- Why the authors used exclusively the cancer cell line HCT116? At least an additional cell line needs to be tested. ?

We used other cancer and normal cell lines in a currently accepted our article "*Effects of Crude Extracts from Medicinal Herbs, Rhazya stricta and Zingiber officinale on Growth and Proliferation of Human Brain Cancer Cell Line in Vitro by Ayman I. Elkady; Rania Abd El Hamid Hussein; Osama A. Abu-Zinadah, BioMed Research International, Volume 2014 (2014) (in Press)*".

2- The informations about PCR contitions (number cycles, annealing and extensions conditions…) are lacking.

The information about PCR run was included in the Material and Method Section in the revised version and more information could be found in our earlier study, references number 33 and 48.

3- The informations about western blot conditions (Amperage, duration……) are lacking.

The gel PAGE was run at 100 V for 2.5 h, a time where the loading buffer reached bottom edge of the gel.

4- No information about antibodies used.

The following antibodies were used: Anti-Caspase 3 antibody (C9598, Sigma), Anti-Caspase 9 antibody (C7729, Sigma), Anti-BCL-2 antibody (SAB45000053, Sigma), Anti-Bax antibody (B3428, Sigma), Anti-Cytochrome c (C9616, Sigma), Anti-p53 C-Terminal (SAB4503001, Sigma), Monoclonal Anti-*β*-Actin (1A5441, Sigma), p21 (**SAB4500065 Sigma**), p27 (**SAB4500067 Sigma**), Anti cyclin D1 C-terminal (M-20) sc-718, (Santa Cruz Biotechnology, Inc), c-Myc C-terminal (C-19) sc-788 (Santa Cruz Biotechnology, Inc), PARP (E4224, Spring BioScience). The secondary antibodies were used: Anti-Rabbit IgG (A4914, Sigma) and Anti-Mouse IgG (A9044, Sigma) and Anti-sheep IgG (A3415, Sigma).

5- How the authors did the densitometric analysis?

The densitometric analysis was done according to on-line protocols, Science Protocols.org (*scienceprotocols.org/**Densitometry**-of-**Gels**.html*).

6- The figures are not correctly reported.

The figures were correctly reported in the revised version.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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