Author’s response to reviews

Title: Antiproliferative and apoptotic effects of proteins from black seeds (Nigella sativa) on human breast MCF-7 cancer cell line.

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Reviewer reports:

Siti Salwa Abd Gani (Reviewer 1):
Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

Ahmed Abdel Moneim (Reviewer 2):

1. Provide the table as a word file not as a jpg file.

Response: As per your kind suggestion, Tables 1 and 2 have been provided in a word file.

2. Phase contrast microscopy images of MCF-7 cells quality is poor and high magnification should be used to show the morphological changes as the authors mentioned in the manuscript.

Response: The image of MCF-7 cells (Figure 3) has been replaced with a better-quality image. We hope that you will find it satisfactory.

3. Western blot analysis should be performed to confirm the PCR results.
Response: We agreed to the reviewer a valuable suggestion. But the primary purpose of this study was to screen the active Nigella sativa protein fractions. In the future, the active fractions will be further purified to study the effect of an individual protein, their synergistic effect, and mechanism using Western blot, flow cytometry, and immunocytochemistry.

4. In vivo study should be conducted to confirm the in vitro study.

Response: We are grateful to the reviewer for a useful suggestion. We agreed that in vivo study will be a valuable addition and would improve the impact of study. However, this preliminary study was conducted to evaluate the anticancer potential of the Nigella sativa proteins in vitro. A study will be designed to conduct in vivo experiments in the future.

Lina Dahabiyyeh (Reviewer 3):

In this manuscript, the authors investigated the anticancer property of the proteins of Nigella sativa, a member of family Ranunculaceae, on MCF-7 cell line. The work is interesting, but I have some minor comments

1. Have the authors evaluated the amount of protein in the crude seed extract (precipitated by ammonium sulphate) using protein quantification kit (i.e, Bradford assay, BCA protein assay kit) to determine % recovery.

Response: Yes, we estimated the amount of crude seed proteins after ammonium sulfate precipitation and gel filtration chromatography peaks using commercially available Bradford assay kit. A paragraph describing the Bradford assay protocol has been added in the methodology section (Page no. 7, line 147)

2. Generally, more details should be added to the methods section. Example; what was the number of fractions collected, volume and amount of protein loaded on gel, Concentrations of crude extract and fractions tested in MTT assay ……

Response: Thank you for the thoughtful suggestion. Corrections have been made accordingly in the methodology section. (Page no. 6, line 142-144) (Page no. 7, line 155-156) (Page no.8, line 169-172)

3. In One-way ANOVA why the authors used Tukey's post hoc and not Dunnett especially that they are comparing to control.

Response: Thank you for the valuable suggestion. We accept that the Dunnett post hoc test is more appropriate for data in which treatment groups are compared to the control. We now have reanalyzed our data using Dunnett as post hoc test and observed a difference in the p values, but the differences were not large enough to influence the overall statistical significance (Page 10, line 231 and page 11 line 232-233)

4. Sentence line 237, page 11, please clarify. 11-18 fractions?

Response: To avoid any possibility of confusion, “11-18 fractions” has been replaced with “peaks (P5, P6, and P7) did not show any protein band” (Page no 11, line 248-249) in the revised manuscript.
5. Page 11 under Analysis of pro-apoptotic, Figure 4, not 3

Response: Corrected (Page no. 12, line 267)

6. In MS identification, what was the minimum number of matched peptides for identification? Was there any protein that was identified with only one or two matched peptides?

Response: To avoid false positive, only proteins with more than two peptides matched were considered for identification. However, few proteins were observed with less than two matched peptides. The information has been added in the methodology (Page no. 10, line 219-220)

7. Why the crude extract (even at high concentration) did not show any cytotoxic effect, although it is supposed to have all the fractions.

Response: There are several reasons one could be, as the crude is a mixture of both active and inactive fractions, the concentration of active proteins (fractions) were not large enough to give cytotoxic effect. Another reason is the synergistic effect of the inactive fractions that may have contributed to suppressing the active fractions.

8. In figure 4 legend, what does it mean the data were normalized with GAPDH?

Response: Data normalization by the housekeeping gene is important for the relative quantification of gene expression by real time PCR. It increases the reliability of the experiment by preventing sample to sample variation. These housekeeping genes (also known as reference gene or internal control) are generally constitutively expressed genes, and ideally their expression should remain same in all experimental conditions such as in control and treatment groups. In our study after analyzing the CT values from both control and treated samples, we selected glyceraldehyde 3-phosphate dehydrogenase (GAPDH) as a reference gene.

9. Try to be consistent (hour or h, Nigella sativa or NS…), abbreviation should be mentioned the first time as pI

Response: The manuscript has been critically revised according to your kind suggestions. Abbreviations have been corrected throughout the manuscript and can be viewed as a track change.

10. Slight mistakes; line 177 remove by, line 232 remove the line 349 belong ....

Response: As suggested, all corrections have been made in the revised manuscript. (Page no. 8, line 188 removed “by”) (Page no. 11, line 243 removed “the”) (Page no. 16, line 356 replaced “belongs” with “are from”)