Author's response to reviews

Title: Antiproliferative activity and induction of apoptotic by ethanolic extract of Alpinia galanga rhizome in human breast carcinoma cell line

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Author's response to reviews: see over
Dear Prof. Tom Rowles,
Senior Executive Editor
BMC Complementary and Alternative Medicine

Thank you for your kind email and helpful comments and suggestion on the language and structure of our manuscript. We have revised the manuscript accordingly, and detailed corrections are listed below point by point:

**Reviewer: 1**

**Point 1:**

**Comments and Suggestions for Authors**

This is a nice paper shedding light on the effect of ethanolic extract of galangal rhizomes to induce cytotoxic in cultured human breast carcinoma cell line in compare to the human fetal lung fibroblast cell line. The authors have also shown that the induction of apoptosis occurs through apoptotic effects of alpinia galangal. I think, this is an interesting work. These data clearly show the extract of alpinia galanga exerts proapoptotic effects in a breast cancer-derived cell line. The introduction establishes a logical basis for the intent and rationale for the experiments. The experimental analyses appear to have been based on standard peer reviewed methods and the statistical analyses seem sound and clearly communicated. The results are clearly presented in the figures. Therefore, I find that this manuscript is acceptable for publication. However, I recommend publication of the paper with appropriate modifications that take into account the following points.

1- While generally written clearly, there are some sentences that need editing. Therefore, I think, the manuscript will require a simple English editing before publications, as several rather minor grammatical and syntax errors are present. This is not a major concern, the authors’ intent was clear.

**Answers to reviewer’s comments.**

Thank you for the comments. We appreciate your kind attention on our manuscript. We have checked the whole manuscript carefully and revised it carefully and we did our best to avoid any mistake including grammar errors or incorrect sentences. We have also asked our colleagues who are skilled authors of English language papers to check again the English and also revised it by a native speaker. We believe that the language is now acceptable for publishing in BMC Complementary and Alternative Medicine.
**Point 2:**

*Comments and Suggestions for Authors*

2- I think, Table-1 is not necessary part of the manuscript. It is better omit the Table-1 and then, add the content of the Table-1 in the result section.

**Answers to reviewer’s comments.**

Thank you for the comments. According to the reviewer’s comment, we omitted the Table-1 then; we added the content of Table-1 in the result section.

**Point 3:**

*Comments and Suggestions for Authors*

3- Material and Methods section, on page 5, Preparation of the galangal Extract, “The preparation was then filtered off through a Gauze mesh and the solvent was by C.” is uncompleted sentence. Please complete the above sentence.

**Answers to reviewer’s comments.**

We are sorry for our mistake. At the Material and Method section, on page 5, the sentence about preparation of the galangal extract, has been corrected as the following:

“The preparation was then filtered off through a Gauze mesh and the solvent was dried by evaporation under reduced pressure at 45°C.”

**Point 4:**

*Comments and Suggestions for Authors*

4- On page 5, Preparation of the galangal Extract, “it was evaporation under reduced pressure at 45 stored in a refrigerator until the experiment” please revise the above sentence.

**Answers to reviewer’s comments.**
Thank you for the comments. According to the above comment, on page 5, preparation of the galangal extract, the sentences revised as the follows:

“The preparation was then filtered off through a Gauze mesh and the solvent was dried by evaporation under reduced pressure at 45°C. The final product yielded 12% w/w dried extract; it was stored in a refrigerator until the experiment.”

Reviewer #2

Point 1:

Comments and Suggestions for Authors

The present study was aimed to evaluate possible anti-cancer effect of alpinia galangal extract on the human breast cancer cells (MCF-7) and the non-malignant cells (MRC-5). The authors incubated the cells with various concentrations of galangal rhizomes extract (0.125, 250, 500, and 750 µg/ml) for 24, 48 h and 72h. They found that the ethanolic extract of galangal rhizomes decreased cell viability in the malignant cells as a concentration- and time dependent manner. Finally, using flow cytometry and Annexin V kit, they have established the induction of apoptosis involved in the cytotoxic effect of ethanolic extract of alpinia galanga in the MCF-7 cell line. I think the manuscript elucidate the results of a good experiment, and the design of the present study was perfect with including the positive and negative control. Therefore, in my opinion, the manuscript have the scientific merit for publication. However, before the paper is published, it should be corrected along the mentioned minor remarks.

1- There is a few spelling errors in the text and it is better that the authors read the manuscript carefully again and revised them: for example: “cell line” (on page 5).

Answers to reviewer’s comments.

Thank you for the comments. We appreciate your valuable sentences on our manuscript. We have checked the whole manuscript carefully and revised it carefully and we did our best to avoid any mistake including grammar errors or incorrect sentences. We have also asked our colleagues who are skilled authors of English language papers to check again the English and also revised it by a native speaker. We believe that the language is now acceptable for publishing in BMC Complementary and Alternative Medicine.

Point 2:

Comments and Suggestions for Authors
2- Table 1 is not necessary; I suggest that it be replaced by adding the data in the text.

Answers to reviewer’s comments.

Thank you for the comments, we added the content of Table- 1 in the result section, therefore according to the reviewer’s comment, we omitted the Table-1.

Point 3:

Comments and Suggestions for Authors

3- The list of references is rather excessive. Suggest trimming down the list to key citations.

Answers to reviewer’s comments.

Thank you for the comment. Since, in introduction and especially in discussion sections, we need express many sentences and for each one, we should refer it to one or some references.

Point 4:

Comments and Suggestions for Authors

4- Symbols and abbreviations are required to be consistent throughout the manuscript.

Answers to reviewer’s comments.

We are sorry for our mistake. We checked the whole manuscript for the symbols and abbreviations. We revised the manuscript, so that, now the symbols and abbreviation are consistent throughout the manuscript.

Point 5:

Comments and Suggestions for Authors

5- Please check symbols (e.g. microlitre; degree Celsius) and subscripts in molecular formula (e.g. CO2, or CH2CL2) in the Materials and Methods and Discussion sections.

Answers to reviewer’s comments.

Thank you for the comments. We checked the throughout the manuscript and revised the symbols and subscripts in molecular formula in the both Methods and Discussion sections.
Point 6:

**Comments and Suggestions for Authors**

6- The discussion section is too long. Discussion needs to be shortened significantly, and it can start from the third paragraph: "Breast cancer is almost resistant...", there is no need to give two long introductory paragraphs in Discussion.

**Answers to reviewer’s comments.**

Thank you for the comments. According the reviewer’s comment, we shortened the discussion section.

Reviewer #3

Point 1:

**Comments and Suggestions for Authors**

1. There is insufficient evidence of the chemical, structural or biological nature of galangal rhizomes extract.

**Answers to reviewer’s comments.**

Thank you for the comments. According to reviewer’s comment, in introduction section, we added the evidence of the chemical, structural and biological nature of galangal rhizomes, then, we added the related references. We added the added the following sentences.

“Phenolic compounds such as flavonoids and phenolic acids are found abundantly in this plant [18]. The dominant components isolated from the rhizomes were galangoisoflavonoid[19], 8-sitosterol diglucosyl caprate[20], methyleugenol, p-coumaryl diacetate, 1′-acetoxyeugenol acetate, trans-p-acetoxycinnamyl alcohol, trans-3,4-dimethoxycinnamyl alcohol, p-hydroxybenzaldehyde, p-hydroxyxynmaldehyde, trans-p-coumaryl alcohol, galangin, trans-p-coumaric acid, and galanganol B [21]. The major phytoconstituents which have been isolated from the rhizomes are acetoxychavicol acetate (ACA) and hydroxychavicol acetate (HCA) [22]. Rhizomes are lowest in fat but richest in carbohydrate [23]. The chemical investigation of A. galanga has led to the isolation of β-caryophyllene (17.95%) and β-selinene (10.56%), terpinen-4-ol[24], 4-allylphenyl acetate and 8-bisabolone, 5-hydroxymethyl furfural (59.9%), benzyl alcohol (57.6%), methylcinnamate (9.4%), 3-phenyl-2-butanone (8.5%) and 1,2-benzenedicarboxylic acid (8.9%)[25]. A new phenylpropanoid, 4,4′-[(2E, 2′E)-bis(prop-2-ene)-1,1′-oxy]- diphenyl-7,7′-diacetata[26], as well as p-coumaryl alcohol-γ-O-methyl ether (CAME) and p-coumaryl
diacetate (CDA), has also been isolated from the plant[27, 28]. Volatile oil of plant contained zerumbone (44.9%), β-farnesene, myrcene and 1,8-cineole, respectively[29, 30]. Bicyclo (4.2.0) oct-1-ene, 7-exoethenyl (58.46%), trans-caryophyllene (7.05%), α-pinene (14.94%) with camphene (2.15%), germacrene (1.78%) and citronellyl acetate (1.41%) were reported in A. galanga as major components [31]. Several authors have studied the anti-inflammatory and analgesic effects of A. galanga in a variety of rheumatological conditions [26, 27, 32, 33]. The extracts of A. galanga showed acetylcholinesterase-inhibitory [34], platelet-activating factor (PAF)-inhibitory [35], antimicrobial [36], antibacterial [37], anti-amoebic [38], antifungal [39], antioxidant [28] and apoptosis [40] activities.”

References:


Point 2:

**Comments and Suggestions for Authors**

2. There is insufficient evidence of the “Growth arrest” effect induced by galangal rhizomes extract. The “Growth arrest” effect should be investigated at least by cell cycle analysis.

**Answers to reviewer’s comments.**
Thank you for the comments. As we noted in our manuscript, in our research, we investigated the potential of galangal rhizomes to induce cytotoxic (antiproliferative) and apoptotic effects in the cultured human breast carcinoma cell line, (MCF-7) in compare with the non-malignant (MRC-5) cells. According to the research, we concentrated the measurements of these parameters by different and specific methods. So that, for assessing the cell viability, we selected the specific method which called the MTT assay, which is based on the conversion of MTT to formazan crystals by mitochondrial dehydrogenises [1]. The result of MTT assay also confirmed by the morphological studies of cell lines using the normal inverted microscope. Then, by using of the Annexin V/PI staining and flow cytometry analysis, we detected the apoptotic cells. In fact, the apoptotic cell death was measured using a flouresin isothiocynate (FITC)-conjugated Annexin V/PI assay followed by flow cytometry. Although, the selected methods in our research (cytotoxic and apoptotic effects) are the main parts of the growth arrest methods in the human breast carcinoma cell line, however, for following of the reviewer’s comment, we replaced the word “Growth arrest” in the title of manuscript into “Antiproliferative activity”, therefore, revised the title of our manuscript as the following:

“Antiproliferative activity and induction of apoptotic by ethanolic extract of Alpinia galanga rhizome in human breast carcinoma cell line”

Point 3:

Comments and Suggestions for Authors

3. There is insufficient evidence of underlying mechanisms of galangal rhizomes extract growth arrest and induction of apoptotic.

Answers to reviewer’s comments.

Thank you for the comments. According to reviewer’s comment, in discussion section, we added the evidence of the chemical, possible underlying mechanisms of galangal rhizomes extract growth arrest and induction of apoptotic, then, we added the related references. We added the added the following sentences.

“Generally, apoptosis can occur via two fundamental pathways: (1) the mitochondrial or intrinsic pathway; and, (2) the death receptor or extrinsic pathway [58]. The intrinsic pathway is triggered by release of mitochondrial proteins, such as cytochrome c, which bind with Apaf-1 and procaspase-9 in a dATP-dependent manner to form the apoptosome [59]. The apoptosome can induce activation of caspase-9, thereby initiating apoptotic caspase cascades [60, 61]. Conversely, the extrinsic pathway is initiated by the interaction of ligands with their respective death receptors, sequentially leading to cleavage of initiator caspase-8. The active caspase-8 cleaves executioner caspase-3, resulting in apoptosis [62]. Activated caspase-9 and -8 further initiate activation of the caspase cascade, leading to
biochemical and morphological changes associated with apoptosis [63, 64]. Thus caspases have been shown to be activated during apoptosis in many cells and play critical roles in both initiation and execution of apoptosis [65]. The intrinsic pathway of apoptosis is regulated by the Bcl-2 family of proteins. Anti-apoptotic (e.g. Bcl-2 and Bcl-xL) and pro-apoptotic (e.g. Bad, Bax and Bak) are two of the major members in Bcl-2 family [66-68]. Anti-apoptotic Bcl-2 and Bcl-xL inhibit apoptosis by sequestering proforms of capsases or by preventing the release of mitochondrial apoptogenic factors [69, 70]. Bad, Bax and Bak inhibit Bcl-2 activity and promote apoptosis [71]. These experimental findings suggest that A.galangal extract induced MCF-7 cells apoptosis however, further detailed investigations of this mechanism are warranted to obtain definite conclusions.”

References:

60. Huang, T.C., 2000. Flora of Taiwan, vol. 5, 2nd ed. Editorial Committee of the Flora of Taiwan, Department of Botany, National Taiwan University, Taipei, Taiwan, pp. 664–691.
Point 4:

Comments and Suggestions for Authors

4. The English grammar needs to be improved throughout the paper. There are many errors of punctuation marks, such as “CO2”-“CO2”, et al..

Answers to reviewer’s comments.

Thank you for the comments. We have checked the whole manuscript carefully and revised it carefully and we did our best to avoid any mistake including grammar errors or punctuation errors.

Point 5:

Comments and Suggestions for Authors

5. The quality of picture should be improved.

Answers to reviewer’s comments.

Thank you for the comments. We did our best to give the good quality of pictures.

Thank you again for great and useful comments and suggestion.

Sincerely Yours,
Saeed Samarghandian