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

Authors:

Saeed Samarghandian (samarghandians@mums.ac.ir)
Jalil Tavakol afshari (TavakolAJ@mums.ac.ir)
Mosa-Al-Reza Hadjizadeh (HadjizadehMr@mums.ac.ir)
Mohadeseh Hosseiny (HosseinyM@mums.ac.ir)

Version: 6 Date: 14 March 2014

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

Thank you very much for your kind email and give us the pleasure comments from the kind reviewers, and the editorial comments. Again, we have revised the manuscript accordingly, and detailed corrections are listed below point by point:

Reviewer: 1

Comments and Suggestions for Authors

Reviewer's report
Title: Antiproliferative activity and induction of apoptotic by ethanolic extract of Alpinia galanga rhizome in human breast carcinoma cell line
Version: 5
Date: 15 February 2014
Reviewer: Tahereh Farkhondeh
Reviewers report:
All requested comment was done well. Therefore, I find that this manuscript is acceptable for publication.
Thank you.
Best Regards,
Tahereh Farkhondeh (Ph.D)
Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.
Declaration of competing interests:
I declare that I have no competing interests to all of the above

Answers to reviewer’s comments.

Thank you for the comments. We really appreciate the kind attention again on our manuscript. After receiving, your previous comments, we did our best to revise the manuscript based on your important comments (point by point), and now, we are very happy to receive your second opinion on our manuscript that after re-checking the manuscript, you found, all your comments and suggestion have been done. Then, you kindly mentioned that our manuscript in current revised form acceptable in for publication.

Thank you again.
Reviewer #2

Comments and Suggestions for Authors

Reviewer’s report

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

Version: 5 Date: 3 March 2014
Reviewer: sediqeh jalali

Reviewer’s report:
I re-checked again the revised manuscript.
I found, they removed the errors and revised the manuscript.
They revised the following points.
1- They corrected the errors in English and grammatical mistakes, the wrong use of a term or spelling mistakes. Now, in the present form of manuscript, I think, its fine for publication in BMC Complementary and Alternative Medicine.
2- As I recommended, they reduced the list of references.
3- They also shortened the discussion section as I mentioned.
4- Therefore, in the revised form, I strongly recommend the manuscript for publication in Your Journal.
Thank you very much.

Answers to reviewer’s comments.

Thank you for the comments. We are express our sincerely thanks for re-checking our manuscript and really appreciate for strongly recommending our manuscript for publication in BMC Complementary and Alternative Medicine. In your previous valuable sentences on our manuscript, we followed the comments, and based on your important comments, we revised it point by point. We really honor to hear that you now believe, we removed all the errors and followed your suggestion till you recommended it for publication.

Thank you again.
Reviewer #3

Comments and Suggestions for Authors

Reviewer’s report
Title: Antiproliferative activity and induction of apoptotic by ethanolic extract of Alpinia galanga rhizome in human breast carcinoma cell line
Version: 5 Date: 24 February 2014
Reviewer: Quanhong Liu

Reviewer’s report:
Comments to the Author
I have re-reviewed the manuscript entitled “Growth arrest and induction of apoptotic by ethanolic extract of Alpinia galanga rhizome in human breast carcinoma cell line” submitted by Samarghandian Saeed, et al.. Although the present paper has improved a lot, there still some questions need to be addressed before its acceptance.

Major Compulsory Revisions
1. The authors ignored my questions in the first comments.
   There is insufficient evidence to support your conclusion on the pro-apoptosis effect of the extract.
2. Please provide some data about the chemical analysis on ethanolic extract of Alpinia galanga rhizome.
3. Why does the author choose the concentration of 125 µg/ml in Figure 2? And from Figure 2, we could see MRC-5 cells were also damaged after 250 µg/ml Alpinia galanga rhizome ethanolic extract incubation for 72 h. How do you explain it?
4. Figure 3 does not tally consistent with the author had described in the paper, please check it.

Level of interest: An article of limited interest
Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Answers to reviewer’s comments.

Thank you for the comments. We are really express our sincerely thanks for re-checking our manuscript and also honestly appreciate the kind attention again on our manuscript. After receiving, your previous comments, we did our best to revise the manuscript based on your important comments (point by point), in your previous valuable sentences on our manuscript, we followed the comments, and based on your important comments, we revised it point by point. We really honor to hear that you also now believe that “present paper has improved a lot”.

We also honor to receive again some valuable suggestions in our manuscript.

Thank you again.
Thank you for the comments. We really appreciate the kind attention again on our manuscript. After receiving, your previous comments, we did our best to revise the manuscript based on your important comments (point by point), and now, we are very happy to receive your second opinion on our manuscript that after re-checking the manuscript, you found, all your comments and suggestion have been done. Then, you kindly mentioned that our manuscript in current revised form acceptable in for publication.

Point 1:

**Comments and Suggestions for Authors**

1. **There is insufficient evidence to support your conclusion on the pro-apoptosis effect of the extract.**

**Answers to reviewer’s comments.**

Thank you for the comments. Although we mentioned in Material and method and results sections that in our study we used the Annexin V-FITC which is the apoptosis detection kit, and the results of the annexin V-FITC confirmed our conclusion on the pro-apoptosis effect of the extract, however, According to reviewer’s comment, in discussion section, we added the evidence of the pro-apoptosis effect of the extract, then, we added the related references. We added the following sentences.

“To study whether the result of MTT assay was due to apoptosis, cells were stained with Annexin V-FITC and PI. The apoptosis evoked by the extract was confirmed by the annexin V–FITC (Figure 3). In the present study, the galangal-induced apoptosis was involved in cell death. Apoptosis is characterized by distinct morphological features including the chromatic condensation, cell and nuclear shrinkage, membrane blabbing, and oligonucleosomal DNA fragmentation (51). As shown in Figures 3 and 4, the Alpinia extract at 250 and 500 mg/ml induced significant cell toxicity in MCF-7 cells in a dose-dependent manner. Therefore, This anti-proliferative effect was due to the induction of apoptosis as shown by the annexin-V-flow cytometric approach. But, apoptosis only partially contributed in this toxicity, and it might be conducted that nonapoptotic cell death can also be involved in the galangal-induced toxicity in these cells. Although the significant of nonapoptotic cell death in chemotherapy remains largely unclear, it is believed that the nonapoptotic cell death is important under conditions in which apoptosis is...
inhibited (52, 53). Overall, this study showed that the alpinia extract may contain bioactive compounds that inhibit the proliferation of breast cancer cell lines (MCF-7) with the involvements of apoptosis or programmed cell death. Further studies are needed to fully recognize the mechanism involved in cell death, alpina extract could be considered as promising chemotherapeutic agent in lung cancer treatment.”

References:

51- Samarghandian s, Shabestari MM: DNA fragmentation and apoptosis induced by safranal in human prostate cancer cell line. Indian J Urol 2013, 29, 177-183.

52- David JM, Owens TA, Barwe SP, Rajasekaran AK: Gramicidin A Induces Metabolic Dysfunction and Energy Depletion Leading to Cell Death in Renal Cell Carcinoma Cells. Mol Cancer Ther 2013, 12, 2296-2307.


Point 2:

Comments and Suggestions for Authors

2. Please provide some data about the chemical analysis on ethanolic extract of Alpinia galanga rhizome.

Answers to reviewer’s comments.

Thank you for the comments. According to the previous and second reviewer’s comment, in introduction section, we added the evidence of the chemical analysis and also the 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-acetoxyccinnamyl alcohol, trans-3,4-dimethoxycinnamyl alcohol, p-hydroxybenzaldehyde, p-hydroxycinnamaldehyde,
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 $\beta$-caryophyllene (17.95%) and $\beta$-selinene (10.56%), terpinen-4-ol[24], 4-allylphenyl acetate and 8-bisabolene, 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’-diaceteta[26], as well as p-coumaryl alcohol-$\gamma$-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%), 6-farnesene, myrcene and 1,8-cineole, respectively[29, 30]. Bicyclo (4.2.0) oct-1-ene, 7-exoethenyl (58.46%), trans-caryophyllene (7.05%), $\alpha$-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 3:

Comments and Suggestions for Authors

3. Why does the author choose the concentration of 125 µg/ml in Figure 2? And from Figure 2, we could see MRC-5 cells were also damaged after 250 µg/ml Alpinia galanga rhizome ethanolic extract incubation for 72 h. How do you explain it?

Answers to reviewer’s comments.

Thank you for the comments. As you can see easily, in figure 2, we only can talk about morphological assay not more, since, it’s only a photo of part of cells by invert microscope!. If you look at figure 3 which is based on our MTT results (Cell viability was measured using the MTT assay), and also in Material and Methods, at those sections, we also clearly noted that we selected the following concentration of the extract: 125, 250, 500, 750 µg/ml. Thus, in figure 2, we would like to know the only morphological aspect of the low concentrations (125 and 250 µg/ml) of the extract for the longest period of exposure.

As we mentioned clearly above, figure 2 only can show the morphological aspect of the cells from different concentrations of the extract, for getting the information about how affect of 250 µg/ml Alpinia galanga rhizome on MRC-5 cells for 72h, only figure 3 (part C), (the data comes from the analytical analysis) shows the cytotoxic effect of the extract. Look at the figure 3 (part 3) doesn’t show a significant damage in the cells after the cells treated with 250 µg/ml Alpinia galanga rhizome ethanolic extract incubation for 72 h.

Point 4:

Comments and Suggestions for Authors

4. Figure 3 does not tally consistent with the author had described in the paper, please check it.

Answers to reviewer’s comments.
Thank you for the comments. We checked it carefully, we believe; figure 3 completely consist with the whole manuscript. If fact, after we got the data of figure 3, we continue our experiment by using the flow cytometry and annexin FITC to know about the mechanism of the acquired data in figure 3. Then we discovered, the one of possible mechanism involved in figure 3 is apoptosis (Figure 4 and 5).

Thank you again.

The editorial comments

*Comments and Suggestions for Authors*

1- Please confirm whether a voucher specimen of the plant material used in your study has been deposited in a publicly available herbarium, and include a statement to this effect in your manuscript. A voucher number should also be included if available.

*Answers to reviewer’s comments.*

Thank you for the comments. According to the editorial comments, we added the following sentences in Material and Methods section.

“The Fresh rhizomes of alpinia galanga used in this study was collected from a private garden in the flowering period in India and identified by botanists in the herbarium of Ferdowsi University of Mashhad and also has been deposited in the herbarium. Voucher specimen is deposited in the specially maintained herbarium, Department of Botany, Ferdowsi University of Mashhad.”

*Comments and Suggestions for Authors*

2- Please include Authors ‘Contributions and Competing Interests sections in your manuscript.

*Answers to reviewer’s comments.*
Thank you for the comments. According to the editorial comments, we added the Author contributions and also the Competing Interest sections in last part of our manuscript before the Reference section.

Thank you again.

Sincerely Yours,

Saeed Samarghandian