Author's response to reviews

Title: Teng-Long-Bu-Zhong-Tang, a Chinese herbal formula, enhances anticancer effects of 5-Fluorouracil in CT26 colon carcinoma

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Author's response to reviews: see over
Dear Mr James Prozenko and Dr Matthias Rostock:

Thank you very much for your email of Mar. 27 regarding the decision of our manuscript (MS: 3881746905965288), entitled “Teng-Long-Bu-Zhong-Tang, a Chinese herbal formula, enhances anticancer effects of 5-Fluorouracil in CT26 colon carcinoma”. The reviewers offered us very good suggestions and comments, which are very helpful for us to revise our manuscript and further investigate. We have revised the manuscript by a point-by-point response to the comments as following:

Response to the comments of Editors:

To the specific comment: Please revise your ethics statement to provide the name of the ethics committee that approved your manuscript.

We have revised the ethics statement as the Longhua Hospital Animal Care and Use Committee.

Response to the comments of the reviewer # 1:

To the specific comment 1: The authors quantify immunohistochemistry data from tumor sections. As tumors are typically heterogeneous, typically several fields of a tumor tissue slide are investigated and cell counts are averaged. This should give a rather representative measure on the whole tissue. How many microscopic fields did the authors assess per slide?

We performed Immunohistochemistry in three tumors in each group and assessed three microscopic fields per slides. We have added those information in figure legends.

To the specific comment 2: In Figure 3, Figure 4 and Figure 6 the authors show immunohistochemistry data of protein expression of cell cycle proteins, XIAP, and surviving as well as cleaved PARP species. For the same reason stated above, this are certainly not the appropriate methods to quantify protein levels in this setting. Western Blot analyses are required to compensate for locally variable protein expression. The authors need to add conclusive Western Blot data to support their findings.
We have detected c-PARP, XIAP, Survivin, p16, p21, pRB and VEGF by western blot and added the results in related figures.

**To the specific comment 3:** Statistical analysis labels have to be added to the figures and information on tumor numbers and statistical tests are required in the figure legends.

We have added statistical analysis labels in related figures and information on tumor numbers and statistical tests in the figure legends.

**To the specific comment 4:** The manuscript needs some language editing for English grammar.

We have revised the language.

**Response to the comments of the reviewer # 2:**

**To the specific comment 1:** Characterization of the Chinese herbal formula. It consists from 8 different herbs. In the present study, there is only a poor description of this remedy. Commonly a TLC (chromatography) from the remedy is presented. How could be compared and characterized the different productions of the remedy? The authors need to present a TLC as supplementary informations.

We have performed GC/MS, and added GC/MS profile of TLBZT as supplementary information.

**To the specific comment 2:** Further in the discussion it will be more important to describe the bioactive components and/or groups in these 8 different herbs and their possible effect regarding the cancer chemoprevention (as authors described e.g. by mistletoe), then description of the role of single herbs in TCM herbal preparations (monarch, minister etc). Including these missing informations, the article will be of interest for more researcher's groups. I recommend authors to describe the cancer chemopreventive effects of single herbs (if such effects were already described; references) at least. Discussion will be more clear and compact.

We have added possible bioactive components information, cancer chemopreventive effects of single herbs.

All those revision are marked with red color.
Once again, I would like to thank you and your reviewers for their constructive suggestions and comments. We hope current revision of our manuscript will be to your satisfaction for publication in *BMC Complementary and Alternative Medicine*.

Please do not hesitate to contact me if there are additional questions.

We are very appreciated at it if you would contact with us by e-mail or Fax mentioned blow.

Sincerely yours,

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