Author's response to reviews

Title: Bu-Zhong-Yi-Qi Pill Alleviate the Chemotherapy-Related Fatigue in 4T1 Murine Breast Cancer Model.

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Author's response to reviews: see over
Dear Editors and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Bu-Zhong-Yi-Qi Pill Alleviate the Chemotherapy-Related Fatigue in 4T1 Murine Breast Cancer Model” (MS Number 1084794984944111). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have taken the comments into consideration in preparing our revision. The responses to the reviewers’ comments and the main corrections in the paper are as following:

**Reviewer: 1**

1. In the background paragraph, author mentioned fatigue is a key reason of patient discontinuation of paclitaxel treated, but the reference 3 seems not related to this statement.


2. In this study, authors tried to use the evaluated of swimming; cytokines changes and production of MDA to prove that the herb of study is beneficial to the chemotherapy-related fatigue; however, weather the change of these parameters is equal to the changes of fatigue, authors should offer more evidences to prove it.

   Response: Swimming until exhaustion is the common experimental protocol used for inducing fatigue in laboratory animals. Researchs have reported that cytokines play important roles in the development of fatigue, and growing evidence indicates that exposure of elevated oxidants is known to cause muscle weakness and accelerate the development of fatigue. This research demostressed that the changes of these markers could be the mechanism of BZYQ in alleviating paclitaxel chemotherapy-related fatigue, but did not stand for be equal to the changes of fatigue completely.
3. The manufacture process of the herbs used in this study should be written in the manuscript.

Response: Bu-Zhong-Yi-Qi pill used in this study was acquired from Wanxi Pharmaceutical Co., Ltd. (Nanyang, China). The contents and the manufacture methods of the drug comply with the pharmacopoeia of China.

4. Is the dosage of herbs that animal taken is equal to the dosage of patient took in clinical treatment? Author should tell us the calculated method of the experimental dosage.

Response: The clinic dosage of the drug is 9g/60kg/d, in this study, the dosage that animal taken is 10x patient’s clinical dosage, same as clinical equivalent dose.

5. In the results of body weight change of mice (Fig 1.), there are some questions should be further discussed:

   A. Why the body weight of group PTX did not increase after the treatment?
   B. Author should compare the differences between PTX+BZYQ group and PTX group.
   C. Authors should discuss the reasons why the treatment of BZYQ was able to increase the body weight of mice.

Response:

   A. Tumor is a kind of wasting disease, and gastrointestinal dysfunctional is one of the serious side effects of PTX, so the body weight of PTX group did not increase.
   B. We have compared the weight between BZYQ+PTX group and PTX group, the BZYQ + PTX group presented a tendency of heavier body weight than the PTX group but there was no significant difference.
   C. In the prior studies, BZYQ has been identified as an effective medication to improve the quality of life and nutritional status, but in our study, we just can assume that BZYQ has the potential effects on increasing body weight because of there was no significant difference between BZYQ+PTX and PTX groups as same as the BZYQ and TC groups. We consider that may be correlated with small sample size, and we will enlarge the sample size to confirm the hypothesis in the next step.
6. In figure 2, author should compare the differences between BZYQ group and TC group in day 12 to day 15. The risk of tumor growth enhanced by the treatment of BZYQ should be discussed from the data of this figure.

Response: In figure 2, the tumor volume of the BZYQ group looked bigger than the TC group at day 12 and day 15, but there was no significant difference between them in statistics. In this study tumors sizes were measured every three days using calipers, the result could be caused by inevitable errors. We used the more exact target-tumor weight to represent the change of tumor.

7. In figure 3, why the result of tumor growth in BZYQ group is different from the result in figure 2?

Response: Tumors were excised from the mice and weighed at the end of the experiment, the weight of tumor was the most exact results to represent the change of tumor. In figure 3, the tumor weight of BZYQ group was lighter than the TC group, but with no statistically significant difference, which is consistent with the result in figure 2.

8. In the results of figure 4, there are some questions to be answered:

A. The survival rate of those treated only by BZYQ is higher than TC group; however, this result seems not compatible with the data of tumor growth, the reason should be discussed.

B. From the data of this figure, we can find that BZYQ plus PTX can prolong the survival rate of mice compared with the use of PTX only, this finding should be an important key of this study and should be put into the conclusion.

Response:

A. Same as the question 7, the result of longer survival rate of BZYQ group than TC group was compatible with the data of tumor growth in fact.

B. In the study, compared with mice in TC group, the median survival time and the average survival time in BZYQ+PTX and PTX groups was significantly prolonged. Though there was no significant difference, the BZYQ + PTX group presented a tendency of longer median survival time than the PTX group.
9. In the results of table 3, the reasons why BZYQ treated along enhanced the production of MDA compared with NC group should be discussed.

Response: Numerous studies has reported that tumor could directly or indirectly produce a state of oxidative stress which may be the reason why the MDA level of the TC and BZYQ groups were higher than the NC group, but with no significant difference between the two groups, the difference of the mechanism of the oxidative stress caused by tumors and chemotherapeutic agents need to be further study.

Minor essential revisions:

1. From the statement of paragraph of discussion, authors cited the references 26~31 and mentioned that cytokines, TNF-alpha, IL-beta and IL-6, play important roles in the development of fatigue; however, some of these references mentioned the cytokines were related to behavior change instead of fatigue.

Response: The references 26~31 we cited in this study are discussing the relationship of cytokines and sickness behaviors, which including fatigue.

Reviewer: 2

1. Please clarify all the number of mice in different time and experiment in the text and figures.

Response: In this study, 150 female BALB/c mice were obtained, 30 mice were randomly chosen as the normal control group, the rest 120 mice were used for establishing tumor grafts model and were randomly divided into 4 groups, in each group, 30 mice randomly divided into 3 subgroups. Tumors sizes of subgroup 1 of each group were measured every three days, the subgroup 2 were taken out from each group for weight-loaded swimming test, and the subgroup 3 in each group were observed for survival analysis.

2. From the figure 5 we can found more significant problem of cancer related fatigue and no effect of BZYQ for that. Why you just focus about chemotherapy related fatigue and the effect of BZYQ is so insignificant?

Response: Fatigue is frequently reported as the common side effect of PTX, and in this study the swimming time of the PTX group were significantly decreasing after
the treatment in week 1 while the swimming time of the TC group did not change obviously. At the same time, the BZYQ +PTX group gradually increased and was longer than the PTX group in week 2 and week 3. There were no significant difference between the BZYQ and the TC groups, but the BZYQ group had a tendency of longer swimming time, lighter tumor weight and longer median survival times than the TC group. So we focus about the chemotherapy related fatigue and the effect of BZYQ.

3. You taken out 10 mice for weight-loaded swimming test every 7 days. Why you just test 10 mice of each group and how you keep the some 10 mice in each test?

Response: The subgroup 2 were taken out from each group for weight-loaded swimming test.

4. You used 10 mice in each group for observed of survival so how many tumors were excised from the mice and weighed at the end of the experiment? What's the number of mice use in the test for tumor weight? How you make choice about this 10 mice in different group?

Response: The subgroup 1 were taken out from each group for tumor volume and weight measurement, and the subgroup 3 in each group were observed for survival analysis.

5. Your will advise to add about the limitation of your study.

Response: The limitation of the study has added.

We appreciate for Editors/Reviewers’ warm work earnestly and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions

Yours sincerely,

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